Comprehensive

BIOCHEMISTRY

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بسم الله الرحمن الرحيم

Dedicated to :

My Wife and Children for their Patience and encouragement untill this book was born.

CONTENTS (VOL. 1, PART II)

ENZYMES

Definition:

These are specialized proteins that accelerate the rate of chemical reactions according to body needs. They are protein catalysts.

Catalysts:

Catalysts are not changed as a result of catalysis. They accelerate the rate at which the reaction reaches equilibrium without changing the equilibrium constant. They perform its action by lowering the energy of activation (lowering barriers to reaction). Therefor a catalyst does not change the thermodynamic properties of the reaction.

General Terms:

1) **Rate of reaction :** The change in amount (moles or grams) of starting materials or products of reaction per unit time (minute or seconds).
2) **Apoenzyme:** Protein part of an enzyme without any cofactor or prosthetic group (inactive).
3) **Cofactors :** Small organic or inorganic molecules required by an apoenzyme to be active. They are loosely bound (Cu for lysine oxidase).
4) **Prosthetic group :** Like cofactors but are tightly bound to apoenzyme.
5) **Holoenzyme** : Apoenzyme + cofactor or prosth. gr. It is the active enzyme.
6) **Substrate :** The substance upon which the enzyme act.
7) **Substrate binding site :** A site on a particular region of an enzyme protein which has certain am. ac. residues that binds to a specific substrate.

8) **Active site :** Contains particular am. ac. residues on the enzyme protein which act as the machinery involved in enzyme action.

9) **Catalytic activity:** The reaction catalyzed by the enzyme:

$$\text{Substrate} \xrightarrow[\text{Cofactors}]{\text{Conzymes}} \text{products}$$

10) **Zymogens (proenzymes):** Inactively synthesized enzymes.

Examples: Pepsinogen, trypsinogen, and prothrombin.

Activated by removal of a polypeptide chain that masks the active site by:

a) Certain agents: hydrogen ion for pepsinogen, enterokinase for trypsinogen.

b) Autocatalytically by the active enzyme itself : pepsin for pepsinogen and trypsin for trypsinogen.

Coenzymes:

1) Specific heat stable low molecular weight organic molecule.
2) Bound either covalently or noncovalently to apoenzymes to form the holoenzyme (prosthetic groups are covalently bound).
3) Examples: **a)** Hydrogen carriers : NAD, NADP, FAD ...... etc. **b)** Groups carriers: Vit B complex (Biotin, thiamin, pyridoxin, folic acid, B_{12}), coenzyme A ......etc.
4) Act as second substrate because always the chemical changes that occur in coenzymes counterbalance those that occur in the substrate. (when substrate is oxidized the coenzyme is reduced).

Enzyme nomenclature:

1) Usually enzyme names have the suffix " **ase**" attached to the name of substrates (glucosidase) or the type of reaction catalyzed (isomerase, adenylcyclase).

2) Sometimes enzymes retain their **"unsystematic"** names when early discovered (pepsin, trypsin, ptyalin ... etc).

3) The IUB (International Union of Biochemists) developed **"systematic"** names in which the suffix "-ase" is attached to a fairly complete description of the chemical reaction calaysed.

Examples :

a) Alcohol dehydrogenase ⟶ alcohol NAD oxidoreductase .

b) Hexokinase ⟶ ATP – D - hexose - 6 - phosphotransferase.

It is to be noted that names adoped by IUB are not in common use. Unsystematic names are recognized & used.

Classification of Enzymes:

Six classes of enzymes are provided by IUB depending on **a)** general type of chemical reaction **b)** type of bond split or formed **c)** type of chemical group removed or transferred.

1) Oxidoreductases : For oxidation reduction reactions between two substrates by removal of hydrogen from one substrate (S_1) to a second substrate (S_2) which may be hydrogen carrier (NAD).

Subclasses:

a) Oxidases: transfer 2 electrons to $O_2 \longrightarrow H_2O_2$.

b) Oxygenases : transfer O_2 into substrates ($A + O_2 \longrightarrow AO_2$)

c) Peroxidases : H_2O_2 + NADH ⟶ NAD + H_2O.

d) Catalase : $H_2O_2 + H_2O_2$ ⟶ $2H_2O + O_2$.

e) Dehydrogenases : transfer electrons to hydrogen carriers.

f) Hydroxylases: introduces one atom of O_2 into substrate while it reduces the other atom to water.

1) **Transferases:** Transfer of functional groups between donors & acceptors.

Subclasses:

a) Aminotransferases (transaminase): transfer of NH_2 gr from one amino acid to an α – keto acid ⟶ new am. ac.

b) Kinases (glucokinase) : transfer P from ATP to NH_2 & OH.

c) Glucosyltransferase (glycogen synthetase) : transfer of activated glucosyl to glycogen primer.

d) Transaldolase & transketolase .

e) Phosphomutases.

3) **Hydrolases:** Special gr. of transferases. The donor is transferred to H_2O ⟶ cleavage of C-O , C-N, O-P and C-S bonds.

Subclasses:

a) Esterases. b) Peptidases. c) Phosphotases.

d) Thiolases. e) Phospholipases. f) Ribonucleases

g) Amidases.

4) **Lyases:** Add or remove the elements of water, ammonia or CO_2.

Subclasses:

a) Decarboxylases b) Aldolases c) Dehydratases.

d) Synthases e) Lyases.

5) Isomerases: Heterogeneous group that catalyze isomerisation of several groups.

Subclasses:

a) Epimerases: inversion at asymmetric carbon. (Xylulose $\rightleftarrows$ Ribulose)

b) Mutases: intermolecular transfer of a group from one carbon to another (phosphoglucomutase).

c) Isomerases:

6) Ligases: Used in synthetic reactions where two molecules are joined using energy released from ATP hydrolysis.

Subclasses:

a) Synthetase : glutamine & acyl CoA synthetases.

b) Pyruvate carboxylase: add CO_2 to pyruvate.

Mechanism of enzyme action :

Enzyme action occurs in steps **First step :** Substrates (S) bind to **substrate biding site** on enzymes. This site becomes the **active site** at which reaction takes place and products (P) are formed.

1) Active or catalytic site.

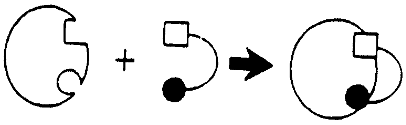
Enzyme regions that bind to substrates specifically.

There may be one or several active sites on one enzyme.

Specificity: depends on chemical groups, their disposition in space and their electrical charge at the site.These chemical groups (-OH, -SH, -NH_3^+& COO) are found on the side chains of am.ac. residues of enzyme protein.

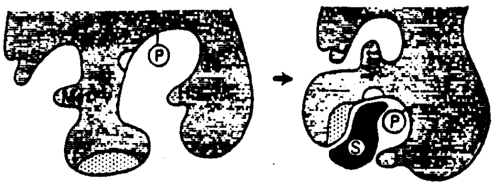
There are two models for specificity of enzymes for its substrate:

a) **Lock- and–key model**: It is an old model in which the enzyme contains a negative impression of the molecular features of the substrate. The binding site is rigid.



Representation of formation of an EnzS complex according to the Fischer template hypothesis.

b) Induced fit model (Kishland fit): Interaction between a substrate with enzyme induces conformational change in the enzyme. The shape of the site is altered so that am. ac. residues and other groups become oriented in space in a correct way for substrate binding . This model is more acceptable and is related to flexibility of the tertiary structure of enzyme protein.



Two-dimensional representation of an induced fit by a conformational change in the protein structure. Note the relative positions of key residues before and after the substrate is bound.

3) Enzyme substrate complex (Michaelis complex).

- Enzymes (E) combine with substrates (S) ⟶ ES complex.
- This combination is by noncovalent hydrophobic bonds leading to:

a) Approximation of all reactants (substrate, coenzymes & cofactors) to each- other & to active site.

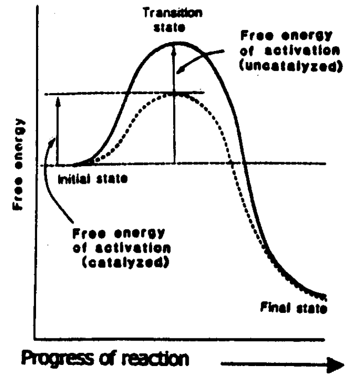
b) Increased local reactant concentration so that all of them come within **bond forming (or bond breaking) distance of one another.**

ES complex dissociates into products(Pand E) at the end of the reaction.

Second step: Enzymes lower activation energy of substrates, leading to more activation of them for the reaction to proceed towards product formation. There are two types of reactions as follows:

1) Uncatalysed chemical reactions :

- Have potential energy barrier namely the activation energy.
- The barrier can be overcome by heat that increases collision of reactants which acquire activation energy & products are formed.
- Transition state of reactants represents reactants in their activated state (or complex) and often they have acquired the energy of activation.



Effect of an enzyme on the activation energy of a reaction.

2) Catalyzed chemical reactions (by enzymes) :

- Enzymes lower activation energy of substrates (reactants).
- Transition state of reactants occurs at a lower activation energy and the
  reaction rate is faster.
- The lower the energy of activation the faster the reaction.
- Enzymes do not change the energies of reactants or products and therefor do not change the equilibrium of the reaction (potential energy difference between the reactants and the products = equilibrium constant).

Specificity of enzymes:

1) **Absolute specificity:** enzymes act on one substrate only (maltase for maltose, glucokinase for glucose, arginase for arginine).
2) **Steriospecificity:** act on only one of sterioisomers (L or D) (L - amino acid oxidase, L-lactate dehydrogenase). However racemase can catalyze interconversion of D & L amino acids.
3) **Groups specificity:** act on the correct bond and the correct part of substrate attached to that bond.

Examples: peptidases act on peptide bonds containing certain groups:

1) **Trypsin :** carboxyl gr. of basic am. ac. (argenine & lysine)

2) **Pepsin :** glutamyl phenylalanine or glutamyl tyrosine bonds.

3) **Chymotrypsin :** carboxyl gr. of aromatic am. ac. (ph. ala, tyro., trypto.)

Kinetics of enzyme action:

It is the study of the rate of change of reactants to products.

A) Definitions:

1) **Velocity:** change of the concentration (mole/L) of substrates or products

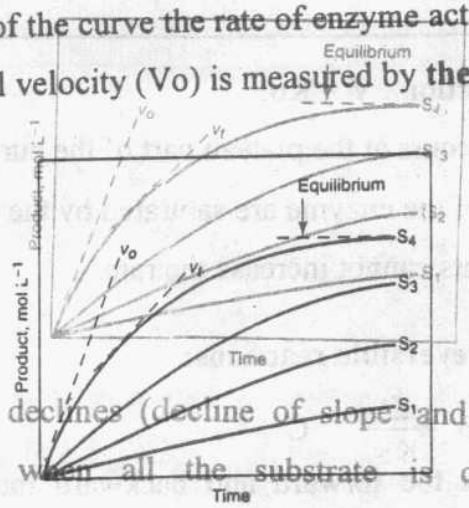
per unit time (**Vo & Vt**) (t = time).

2) **Rate :** change in total quantity of substrate or products (mole or grams) per unit time.

B) Initial reaction velocity (Vo) :

- It is the velocity at zero time.
- A hyperbolic curve will be obtained when an enzyme react with excess substrate and the amount of product is plotted against time (rate of enz. action).

- At the linear part of the curve the rate of enzyme activity increases very rapidly and initial velocity (Vo) is measured by **the slope of the curve near zero time.**



- Then the curve declines (decline of slope and velocity – Vt.) and reaches plateau when all the substrate is changed to products (equilibrium) .
- Vo is characteristic of every enzyme and increases with increase of substrate concentration.
- Rate equations (or velocity equations):

a) First order reaction : $A \xrightarrow{K} P$ $[V = K(A)]$

b) Second order reaction: $A + B \xrightarrow{K} C$ $[V = K(A)(B)]$

V = velocity

A & B = reactants (substrates)

P & C = products

K = rate constant.

Thus the velocity of a reaction could be expressed as substrate concentration. When substrate concentration increases the velocity or rate of reaction increases.

Many biological processes proceed under first order condition. Furthermore drugs are cleared from blood in peripheral tissues by first order process.

c) Zero order reaction : $V = Ko$.

This reaction occurs at the plateau part of the curve at which all catalytic sites of the enzyme are saturated by the substrate & addition of more reactants cannot increase the rate.

- **Equations of reversible reactions:**

$$A + B \underset{K_2}{\overset{k_1}{\rightleftharpoons}} C$$

At equilibrium the forward and backward rates of reaction are the same and the overall conc. of substrate & products do not change with time.

$$Keq = \frac{K_1}{K_2} = \frac{C}{(A)(B)}$$

Keq = equilibrium constant (a thermodynamic state).

K_1 & K_2 = rate constants (kinetic expression related to velocity).

- **Enzyme activity** = micromoles (μ mole) of substrate converted to product per minute (1 unit = μ mole / min).

Catalytic constant = unit of activity per mole of enzyme (μ mole / min / mole of enzyme).

- **Maximum velocity** (V max) : the velocity obtained after substrate saturation of the enzyme under specified conditions of temperature, pH and ion strength. V max is a constant for every enzyme.

C)Michaelis – Menten equation :

-It describes how enzymes interact with substrates.

-Initial velocity (Vo) is dependent on amount of substrate and enzyme concentration. Both more substrate (with fixed enzyme conc.) and more enzyme (fixed substrate conc.) concentrations will increase Vo.

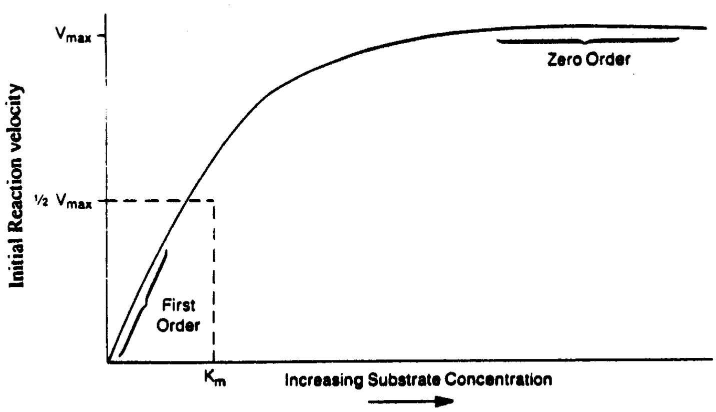
- **Michaelis – Menten curve (Hyperbolic curve):**

a) With fixed amount of enzyme, as the concentration of substrate increases the initial velocity (Vo) increases and more and more enzyme active sites are bound to substrate.

b) Maximum velocity (Vmax) reflects saturation of all active sites in the enzyme (Fixed amount) by substrate.

c) Vmax / 2 or ½ Vmax = half-maximum velocity.

d) Km **(Michaelis constant)** is the concentration of substrate at ½ Vmax.



- **Michaelis Menten equation:**

a) Depends on a general equation of enzyme action based on the presence of enzyme substrate complex (ES).

$$E + S \underset{K_2}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_3} E + P$$

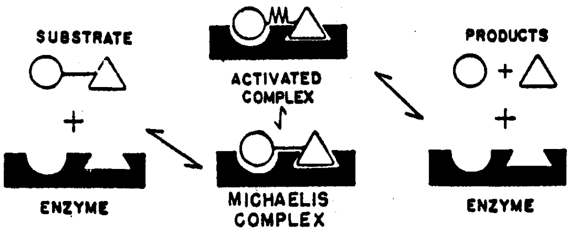
(E = enzyme, S = substrate, ES = enzyme substrate complex, K_1, K_2 & K_3 = rate constants).

b) ES complex (Michaelis complex) is characterized by:

1) During initial phase of reaction: ES remains constant.

2) During saturation condition: all E becomes ES.

3) At maximal product formation: all E is in ES form.



Enzyme action

c) Vmax :

Generally $V = K(A)$ (A is the substrate).

Thus $V_{max} = K_3(ES)$ (ES is comparable to A).

d) Km (Michaelis constant):

- It is obtained from rate constants during initial phase of reaction or at the steady state condition when the velocity of ES complex formation (V_1) equals the sum of velocities of its breakdown (V_2 & V_3).

Or :

$$V_1 = V_2 + V_3$$

$$K_1 (E) (S) = K_2 (ES) + k_3 (ES)$$

$$K_1 (E) (S) = (K_2 + K_3) (ES).$$

$$\frac{(E)(S)}{(ES)} = \frac{K_2 + K_3}{K_1} = Km$$

Or :

$$Km = \frac{K_2 + K_3}{K_1} = \text{(Michaelis constant).}$$

d) Michaelis – Menten equation :

- Obtained after algebraic manipulation as follows.

$$V = \frac{(V\,max)\,(s)}{Km + (s)}$$

- Importance of equation:

1) A plot of Vo versus S gives a hyper bolic curve.

2) Vmax is determined.

3) Km value is determined (μ mole /L)

4) Importance of Km value :

a) It is equal to the concentration of substrate at ½ Vmax.

b) It is a constant characteristic of an enzyme and a particular substrate.

c) It does not vary with concentration of enzyme.

d) It reflects the affinity of an enzyme and its substrate.

e) Low km values mean high affinity and high km values mean low affinity of substrate to enzyme.

Glucokinase has less affinity to glucose (Km = 10) than Hexokinase (Km = 0.1) .

5) At ½ Vmax: Km = S

$$½\ Vamx = \frac{V\,max(s)}{Km + (s)}$$

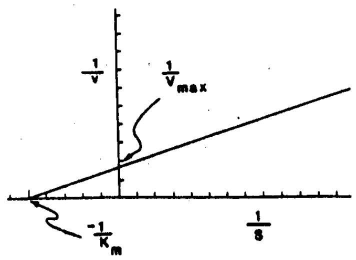
$$Km + (S) = \frac{2\,Vmax\,(s)}{Vmax} = V\,max(s)$$

$$Km = (S)$$

D) Lineweaver Burk equation:

1) More accurate determination of Vmax & Km values of an enzyme.

2) The new equation is developed by **reciprocal of Michaelis – Menten equation:-**



LINEWEAVER – BURKE PLOT.

$$\frac{1}{V} = \frac{K_m}{V\max} \times \frac{1}{(S)} + \frac{1}{V\max}$$

3) Straight line is produced after plotting the $\frac{1}{V}$ versus $\frac{1}{S}$ (this line is called Lineweaver – Burk plot).

4) The intercept on the x axis is equal to $\frac{-1}{Km}$

5) The intercept on the Ý axis is equal to $\frac{1}{V\max}$

Inhibition of enzyme activity:

A) Nonspecific inhibitors :

These are agents which denature the enzyme protein or block specific groups in the enzyme.

1) Sulfhydryl inhibition :

Many enzymes contain SH gr. of cysteine. Agents that block -SH gr. will inhibit ES complex formation followd by decreased enzyme activity.

Examples :

a) Potassium ferricyanide : convert SH to disulfide by oxidation reaction.

$$2\text{-SH} + O \longrightarrow \text{-S-S-} + H_2O$$

b) Iodoacetic acid (alkylating agent) : will ackylate the enzyme.

c) Salts of heavy metals (Hg): form mercaptides

d) War gas (lewisite) : treatment by giving BAL (British Anti Lewisite).

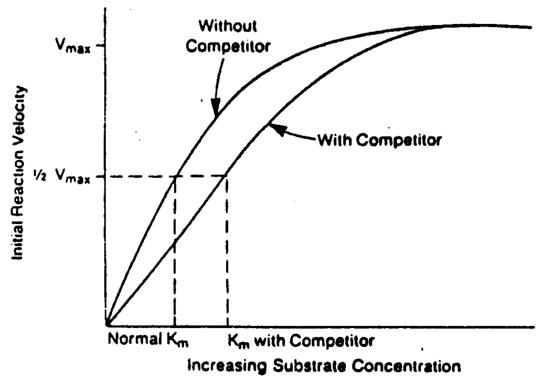
2) Denaturing agents as physical agents (x-ray, heat & UV light & repeated freezing & thawing) or chemical (strong acids & alkalies ... etc).

B) Specific inhibitors:

Exert their actions on only one enzyme or related enzymes.

1) Competitive inhibition (metabolic antagonists):

- Due to structural similarity between the inhibitor & the substrate.
- The inhibitor reacts reversibly with the active site of an enzyme which inhibits ES complex formation followed by decreased enzyme activity.
- Competitive inhibition is **reversible**.
- Both inhibitor & substrate compete with each other for the active site.



Reaction curve showing the effect of a competitive inhibitor.

- The rate of the reaction depends on the ratio of the concentration of inhibitor to that of substrate.
- Addition of more substrate will decrease inhibition.
- Competitive inhibitors will increase the Km value without change of V-max of the enzyme reaction.

<u>Examples</u>: Due to structural similarity between:

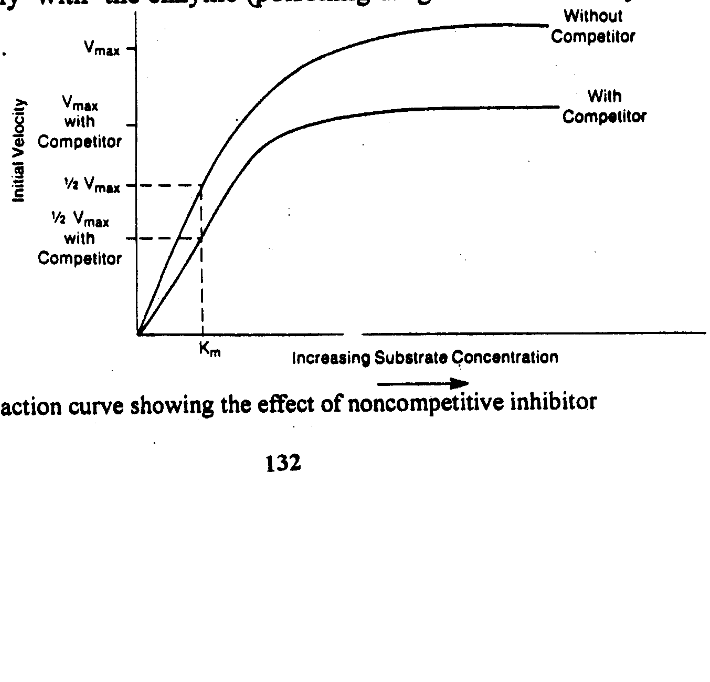
a) Succinate & malonate: inhibition of succinate dehydrogenase.

b) Allopurinol & hypoxanthine: inhibition of xanthine oxidase leading to decreased uric acid formation (allopurinol is used for treatment of gout).

c) Dicumarol & Vit K: inhibition of prothrombin synthesis (dicumarol is used as an anticoagulant).

2) Noncompetitive inhibition:

- Related to concentration of inhibitors (not related to structure of inhibitor or substrate conc.).
- The inhibitor binds at a site other than that for active site.
- The inhibitor combines with the E & ES so that the enzyme is removed from the reaction.
- Vmax is reduced without change of Km.
- It is reversible only after exhaustive dialysis if the inhibitor did not react covalently with the enzyme (poisoning drugs react covalently with the enzyme).



Reaction curve showing the effect of noncompetitive inhibitor

Examples: Inhibitors blocking activators & coenzymes:

a) Fluorides precipitate Ca^{++} & Mg^{++} ions needed by the enzyme (prevent blood clotting).

b) Cyanides & CO block iron of heme in cytochrome oxidae.

c) Cyanides combine with pyridoxal phosphate (inhibit decarboxylases).

3) Allosteric inhibitors:

- These are negative allosteric modifiers involved in many metabolic pathways.
- (Refer to allosteric regulation of enzyme activity.)

Factors affecting enzyme activity:

A) Substrate concentration:

- Refer to Michaelis-Menten equation & curve (Vmax & Km values).

B) Enzyme concentration:

- When an increasing amount of an enzyme is added to excess amount of substrate, the initial velocity doubles as the concentration of the enzyme doubles.
- Thus the velocity of an enzyme reaction is dependent on both substrate and enzyme concentrations.

C) Concentration of inhibitors:

- There is an inverse relationship between concentration of inhibitor and velocity of enzyme reaction.

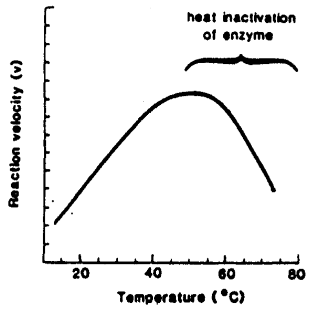
(Refer to enzyme inhibitors section).

D) Allosteric modifiers or modulators:

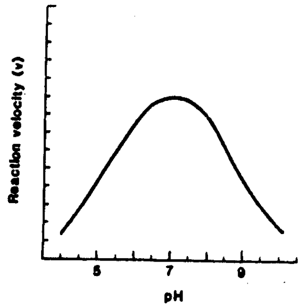
(Refer to allosteric control of enzyme activity) (positive & negative allosteric modifiers).

E) Temperature:

- Rise of temperature increases collision & movement between substrate and enzyme leading to increase in velocity of enzyme reaction.
- At 0°C all enzyme activity is inhibited (V = zero).
- Rise of temperature will increase V and reaches maximum level at optimum temperature (37°C for animals) followed by decline of V with further increase of temperature. V = near zero at 70°C where all the enzyme protein is denatured.
- The rate of many biologic processes show a two fold increase in activity for every 10°C temperature rise (Temperatue cofficient "Q"$_{10}$ = 2).



Effect of temperature on an enzyme-catalyzed reaction



Efect of pH on an enzyme-catalyzed reaction

F) pH:

- H^+ is required to protonate certain groups in enzyme and substrate structurs in order to interact.
- As pH increases the velocity is increased until an optimum pH is reached (pH = 7.0) at which V is at maximum level. Rise of pH above optimum level will decrease V. At about pH 10 all the enzyme protein is denatured.

- Optimum pH for enzymes varies widely:
  Pepsin = 2, amylase = 7, trypsin = 8.
- Enzyme activity usually stops 2 pH units away from optimum pH.

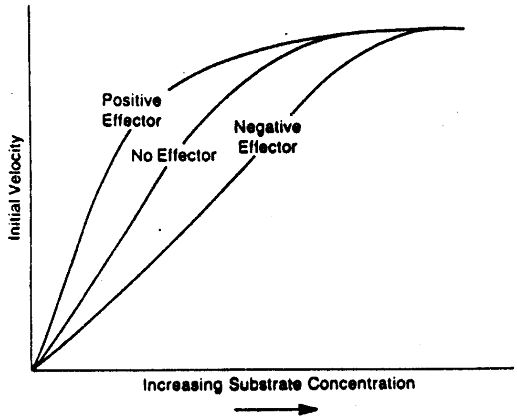
Allosteric control of enzyme activity:

A) It is the modulation of enzyme activity by small molecules (ligands) that bind to **allosteric sites** on enzyme proteins and not related to competitive or noncompetitive inhibitors.

B) Ligands: 1). Are small organic molecules or low molecular weight proteins.
2) Can be activators or inhibitors. 3) Not changed as a result of enzyme action. They are just modulators, effectors or modifiers.

C) Allosterc site:
- Allo = other.
- It is a unique region of the enzyme quite different from active site or substrate binding site.
- Binding of allosteric effectors or modifiers (ligands) to allosteric site causes conformational change of the enzyme so that the affinity for the substrate changes.

D) Allosteric modifiers: They are two types:
1) Positive allosteric modifiers or activators: Allosteric effector increases enzyme affinity (binding) to substrates at the active site.
2) Negative allosteric modifiers or inhibitors: Allosteric effector decreases enzyme affinity (binding) to substrates at the active site.



Kinetic effect of a positive or negative allosteric effector.

Example:

It is one type of feed back inhibitory mechanisms for regulation of enzyme activity in metabolic pathways. The end product in a series of reactions will allosterically inhibit an early enzyme in that series. Excess ATP production during glycolysis leads to ATP allosteric inhibition of phosphofructokinase enzyme, an early enzyme in glycolytic series of reactions.

E) Sigmoidal Michaelis - Menten curve instead of hyperbolic:

- Negative effector shifts the curve to the right (High km).
- Positive effector shifts the curve to the left (low km).

Regulation of enzyme activity:

Regulation of enzyme activity is the study of how enzymes are integrated into a metabolic pathway and the interrelationship of the products of one pathway with the metabolic activity of other pathways.

Definitions:

A **key enzyme** of a certain metabolic pathway has one or both of the following characters:

1) Low V_{max} : **rate limiting enzyme.**

2) Catalyze the first irreversible step in a pathway (committed step).

Regulation:

The rate limiting enzymes and those catalyzing rate limiting or committed steps are regulated by:

A) Amount of enzyme:

1)The de novo synthesis of enzymes (protein biosynthesis) is affected by regulation of **gene expression** of the specific enzyme by either **repression** (inhibition) or **derepression** (stimulation) of gene expression.

Example:

Glucose represses de novo synthesis of pyruvate carboxykinase (rate limiting enzyme in conversion of pyruvate to glucose in gluconeogenesis).

2) Half life of enzyme (the time after which the enzyme is present as half of its amount). Enzymes with short half life are rate limiting.

Example:

Pyruvate carboxykinase has half life = 5 hours.

B) Modification of existing enzymes:

1) Feedback ihibition:

Enzyme activity is inhibited by end products (shutdown of change of substrates to products because the latter is in excess amount).

- Km value increases above the levels of substrates & the velocity decreases.
- Inhibition occurs by either of two ways:
  - a) Competitive : The product competes with the substrate for the active site .
  - b) Allosteric : The product initeracts with allosteric site.

2) Covalent modification :

It is a reversible process by which an enzyme is covalently bound to certain groups which leads to interconvertible active & inactive forms of enzymes.

Examples :

a) Phosphorylation – dephosphorylation of enzymes (as in glycogen metabolism) by various protein kinases (bind P) and phosphatases (remove P). ATP is the source of P which binds to – OH group of serine or threonine of enzyme portein by a covalent bond.

b) Methylation & demethylation or acetylation & deacetylation.

Isoenzymes (isozymes):

A) Definition :

Enzymes that catalyze the same reaction but migrate differently on electrophoresis & are immunologically different.

B) Mechanism of formation :

The arrangement of subunits arising from two different genetic loci in different combinations to form the active polymeric enzyme.

C) Examples:

1) Creatine kinase isozymes (CK):

- Occur as a dimer (2 subunits) : brain (B) & muscle (M) types.
- Brain has B, muscles have M and heart has MB subunits.
- Other tissues have variable amounts of B B & M M isozymes.
- Increased in patients with mycoardial infarction (CK MB).

2) Lactate dehydrogenase isozymes:

- Occur as a dimer (2 subunits): heart (H) & muscle (M) types.
- The two subunits are combined in five different combinations:

a) LDH_1 : heart & RBCs (H H H H).

b) LDH_2: heart & RBCs (H H H M).

c) LDH_3: Brain & Kidney (H H M M).

d) LDH_4: Liver & Skeletal muscles (H H M M).

e) LDH_5: Liver & Skeletal muscles (M M M M)

- Increased in patients with liver (hepatitis), heart (infarction) & muscle (myopathy) diseases.

VITAMINS

Definition :

Organic compounds, present in food and required in minute amounts for a variety of biochemical functions for normal growth. They do not enter into the tissue structure and do not undergo degradation for purposes of providing energy.

Classification & General characteristics :

A) Water soluble vitamins : B-Complex & Vit C.

- Thiamin (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxin (B_6), biotin, cobalamin (B_{12}), folic acid.
- They are extracted in water of cooked food, easily absorbed from intestines without the help of bile salts, not stored in the body, easily excreted in urine and therefor rarely accumulate in toxic concentration and most are synthesized by intestinal bacteria.

B) Fat soluble vitamins : Vit A, D, K & E.

- Associated with dietary fat (they are apolar hydrophobic molecules), absorbed only when fats are absorbed in the small intestine with the help of bile salts, transported in blood (like apolar lipid) in lipoproteins or attached to specific binding protein, cannot be excreted in urine & can be stored in the liver and therefor manifestation of deficiency appears late and toxic manifestations are common and not synthesized by intestinal bacteria (except vit K).

VITAMINS OF THE B COMPLEX

Common properties :

- Soluble in water
- Most of them are :

  a) destroyed by light (only niacin is stable).

  b) destroyed in alkaline medium (only niacin is stable).

  c) stable in acid medium.

  d) heat stable (only Vit B_1 & B_2 are heat labile).

Common sources :

They are widely distributed in plants & animals :

A) Animal sources :

- Meat, liver, heart, fish, milk & eggs.

B) Plant kingdom sources :

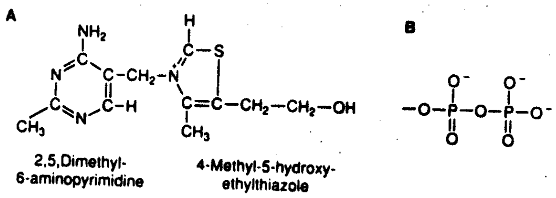
- Seed, leaf, root, stem & fruit of plants.
- Cereal grains : Vit B complex is concentrated in its outer germ & bran layer (e.g rice polishings). Therefor, whole- cereal flour & bread are rich sources while white flour, white bread & polished rice are poor sources.
- Killed yeast, peas, beans, nuts, prunes, legumes and green leafy vegetables are good sources.
- Intestinal bacteria are good source also.

THIAMIN (B_1)

Chemistry :

1) Consists of pyrimidine and thiazole rings joined by methylene group (-CH-).

2) Thiamine pyrophosphate (TPP) is the active form of the vitamin which is formed in the liver & brain by an ATP – dependent thiamine diphosphotransferase.



Thiamin. ***A:*** The free vitamin. ***B:*** In thiamin diphosphate, the -OH group is replaced by pyrophosphate.

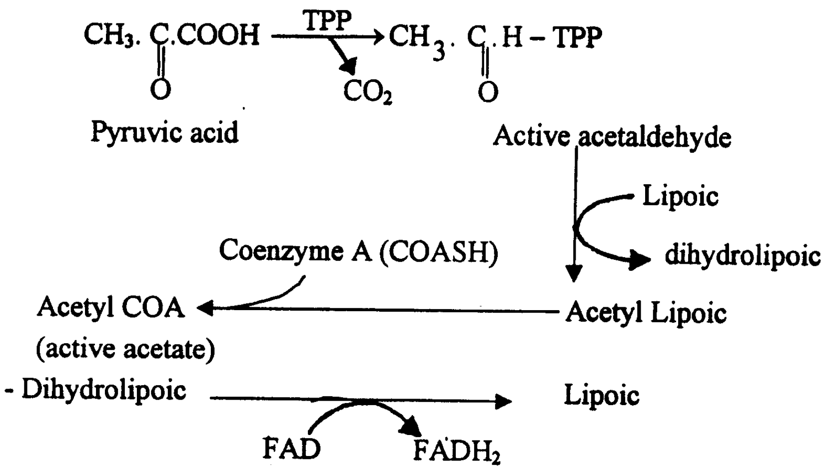
Functions :

It is a coenzyme in oxidative decarboxylation & transketolase reactions.

A) Oxidative decarboxylation of α-Keto acids (pyruvic, α-ketoglutaric) (TPP is named "cocarboxylase") by enzymes present in the mitochondria :

1) Oxidative decarboxylation of pyruvic acid by the pyruvate dehydrogenase complex enzyme which is a multienzyme complex formed of 5-coenzymes (TPP, Lipoic acid, NAD, FAD & Coenzyme A).

a) The reaction starts by the condensation of pyruvic acid with the thiazole ring of TPP and release of CO_2 + active acetaldehyde.



- $FADH_2$ + NAD ⟶ NADH + H

- The H of NADH + H is transferred to the respiratory chain in the mitochonchria for ATP production.

b) Active acetate is formed in the mitochondria in the krebs cycle during aerobic glucose oxidation.

2) Oxidative decarboxylation of α-Ketoglutarate by a similar multienzyme complex like that of pyruvate dehydrogenase complex.

3) Oxidative decarboxylation of the α- keto acid derivatives of the branched chain amino acids (Leucine, isolecrcine & valine) by branched chain α-keto acid dehydrogenase which is similar to the mutlienzyme pyruvate dehydrogenase complex.

α- Ketoglutarate ⟶ Succinyl-COA + CO_2.

B) Transketolation reaction :

- It occurs in pentose phosphate pathway which is an alternate route for glucose oxidation in the cytosol.
- TPP is a coenzyme for transketolase enzyme which catalyzes the transfer of 2-carbon units from xylulose 5 phosphate to ribose 5

phosphate producing the 7 carbon ketose sedoheptulose and glyceraldehyde 3P.

$$\text{Xylulose 5 P} + \text{Ribose 5 P} \xrightarrow[\text{TPP}]{} \text{Sedoheptulose 7 P.} + \text{Glyceraldehyde 3 P}$$

Deficiency :

A) Cause of deficiency : By diets rich in carbohydrate but deficient in thiamin like polished rice or highly refined foods such as sugar and white flour acting as the main food source.

B) Disease : Thiamine deficiency causes a disease named **"beriberi"** which was endemic in the far east during periods of maluntrition (postwar) and characterized by :

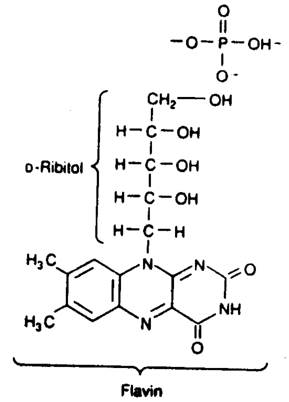
1) Early symptoms of peripheral neuropathy, exhaustion & anorexia.
2) Late manifestations include congestive heart failure and edema (Wet beriberi), muscular degeneration & encephalopathy.

All these manifestions are due to accumulation of pyruvate, pentose and α-keto acids derived from branched amino acids.

RIBOFLAVIN (B_2)

Chemistry :

1) Consists of heterocyclic isoalloxazine ring attached to ribitol (sugar alcohol) forming an orange yellow compound.



Riboflavin. In riboflavin phosphate (flavin mononucleotide, FMN), the -OH is replaced by phosphate.

2) Active riboflavin include :

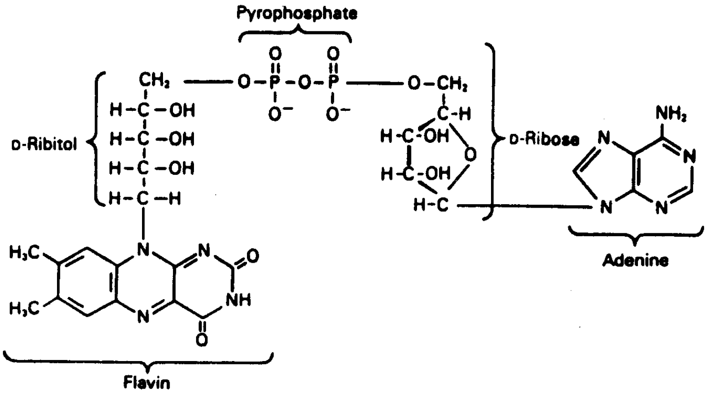
a) Riboflavin mononucleotide (FMN) = Riboflavin + phosphate

b) Riboflavin adenine dinucleotide (FAD) = FMN + adenosine monophosphate.

ATP + Riboflavin ⟶ FMN + ADP

↓ ATP

Pyrophosphate (PPi) + FAD



Flavin adenine dinucleotide (FAD).

Function :

- FMN & FAD are the prosthetic group of oxidoreductuse enzymes known as **flavoproteins.**

- Flavoproteins + cofactors (molybdenum & iron) are known as **metalloflavoprotein.**

- FMN & FAD are coenzymes that act as hydrogen carriers by reversibly reducing the isoalloxazine ring by H atoms yielding the reduced forms FMN H_2 & $FADH_2$.

- Flavoprotein enzymes include :

1) L-α - amino acid oxidase : for amino acid oxidation.

   L-α - amino acid + FMN ⟶ α - keto acid + NH3 + FMN H_2 .

2) D-amino acid oxidase.
3) Xanthine oxidase : in purine degradaton into uric acid.
4) Succinate dehydrogenase : citric acid (Krebs) cycle.

   Succinate + FAD ⟶ Fumarate + $FADH_2$.

5) Acyl COA dehydrogenase
6) Dihydrolipoyl dehydrogenase : in oxidative decarboxylation of pyruvate & α-ketoglutarate.
7) NADH dehydrogenase : in the respiratory chain and contain FMN that transfer electrons from NAD to coenzyme Q (COQ).

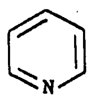
Deficiency :

1) Nonfatal diseases like stomatitis, glossitis, seborrhea & photophobia.
2) Deficiency might occur to neoborn infants who receive phototherapy to treat neonatal hyperbilirubinaemia due to Vit B_2 light sensitivity.

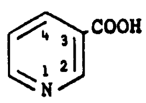
NIACIN (Nicotinic Acid)

Chemistry :

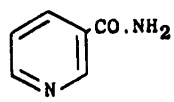
1) Nicotinic acid is pyridine 3-carboxylic acid.
2) Nicotinic acid and its amide (nicotinamide) are named niacin and act as the source of the vitamin in the diet.



Pyridine



Nicotinic acid



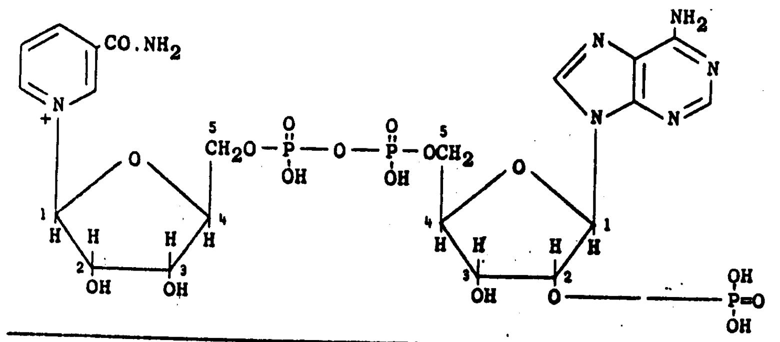
Nicotinamide

3) Tryptophan is the precursor of nicotinic acid in plants and animal species including man. Intestinal bacteria can form nicotinic acid from tryptophan and therefor supplement dietary and animal tissue source of the vitamin.

4) Nicotinamide in the body is deaminated into nicotinic acid which is transformed to NAD & NADP as follows :

a. Nicotinic acid + phosphoribosyl pyrophosphate + ATP + glutamine $\rightarrow$ nicotinamide adenine dinucleotide (NAD).

b. NAD + P $\rightarrow$ nicotinamide adenine dinucleotide phosphate (NADP)



NAD^+

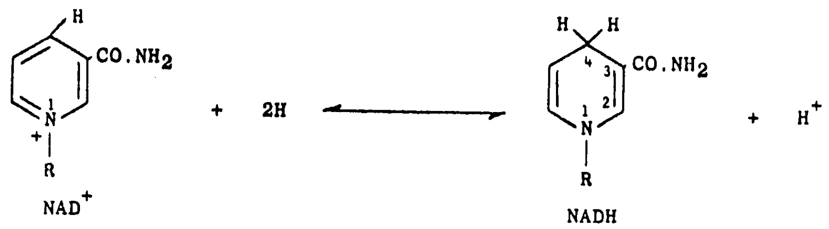
$NADP^+$

5) NAD & NADP are the active forms of niacin.

Function :

- NAD & NADP act as coenzymes in oxidaton - reduction reactions which occur by reversible additon of a hydride ion (H^+) to the pyridine ring at C_4 while N_1 of the ring accepts an electron from another hydrogen atom with the generation of free hydrogen ion (H^+).

$$NAD^+ + AH_2 \longleftrightarrow NADH + H^+ + A$$



- Enzymes that have NAD & NADP coenzymes are key enzymes in carbohydrate, lipid and amino acid metabolism. They include :

A) NAD linked dehydrogenases :

1) Lactate dehydrogenase : In anaerobic oxidation of glucose in the cytosol.

2) Isocitrate dehydrogenase & malate dehydrogenase :

   In aerobic oxidation of glucose in citric acid cycle (Krebs cycle) in the mitochondria.

3) Glutamate dehydrogenase & phenylalanine hydroxylase : In oxidation reaction of amino acids.

4) Hydroxymethylaglutaryl - COA (HMG - COA) reductase : In cholesterol synthesis in the microsomes.

B) NADP linked dehydrogenases :

- Glucose - 6-phosphate dehydrogenase : In pentose phosphate pathway for reductive synthesis of pentoses from glucose in the cytosol.

Deficiency :

- Niacin deficiency occurs when diet is poor in both niacin and tryptophan (every 60 mg tryptophan generate 1 mg niacin).
- Niacin deficiency occurs in populations dependant on **maize** as the main food **(staple food)** which is deficient in tryptophan and niacin is present in bound unavailable form **(niacytin)**.
- **Pellagra :**
  1) Disease due to deficiency of both niacin & Vit B_6. (Vit B6 or pyrodoxal phosphate is involved as a cofactor for the synthesis of NAD from tryptophan).
  2) Manifestations : Dermatitis, diarrhoea, dementia then death (4 D's).
     - Skin inflammation (Dermatitis) occurs in areas exposed to sun light (face, neck, wrists, forearm ....etc).
     - Diarrhoea is due to inflammation of the gastrointestinal tract.
     - Dementia = depression & loss of mental power.
  3) Diagnosis : By two methods :

     a) Clinical response to administration of adequate dose of nicotinate.

     b) Urinary estimation of N-methylnicotinamide which is diminished.

Therapeutic Value :

- Nicotinic acid is used for lowering plasma cholesterol in patients with hyperlipidaemia (type II). Niacin inhibits lipolysis ⟶ decrease in free FF ⟶ less formation of cholesterol ⟶ less formation of Lipoproteins (VLDL & LDL).

PANTOTHENIC ACID

Chemistry : Formed by combination of pantoic acid (α-dihydroxy, β-dimethyl butyric acid) through amide linkage to β-alanine.

$$CH_2OH.\underset{CH_3}{\overset{CH_3}{C}}.CHOH.CO - NH.CH_2.CH_2.COOH$$

Pantoic acid β-Alanine

- α- ketoisovaleric acid ⟶ pantoic acid.
- Aspartic acid ⟶ β-alanine +CO_2.
- β-alanine + pantoic acid ⟶ pantothenic acid.

Function :

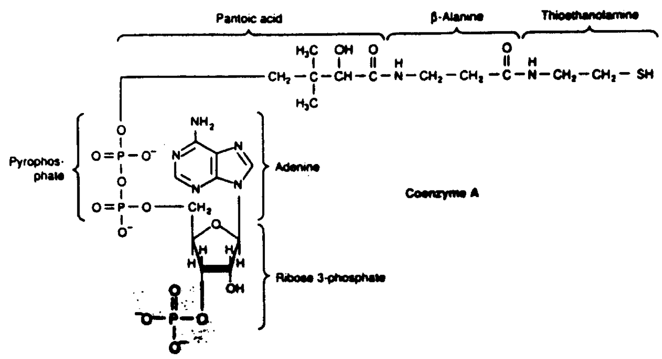
Pantothenic acid is present in tissues in the form of a coenzyme called coenzyme A (COA) (A = acylation) and acyle carries protein (ACP).

A) Coenzyme A :

1) Formed from pantothenic acid joined on one hand to adenosine (by pyrophosphate bridge) and on the other hand to thioethanolamine(by peptide bond).

   (Adenosine + pantothenic acid + thioethanolamine)

   - The terminal thiol group (SH) is the active center of COA that binds acyl compounds (like acetate + COA → acetyl COA) & the coenzyme is abbreviated **COASH**.



2) Function of COA : It acts as acyl carrier in the following reactions :

a) COA + acetate → acetyl COA which is involved in citric acid cycle, fatty acid synthesis & oxidation, cholesterol synthesis..... etc.

b) COA + succinate → succinyl COA which enters in heme synthesis (of hemoglobin).

c) Acetylation of drugs (acetyl COA - drug) → detoxication.

B) Acyl carrier protein (ACP)

1) Structure :

- It is a protein containing a prosthetic group formed of phosphate + pantothenic acid + thioethanolamine.
- The serine residue of the protein is connected to the phosphate of the prosthetic group by ester linkage.

2) Function :

- ACP is **part of fatty acid synthase** which is a multienzyme polypeptide complex for fatty acid synthesis in the cytosol.

- The terminal thiol group (SH) is the active center of ACP during fatty acid synthesis.

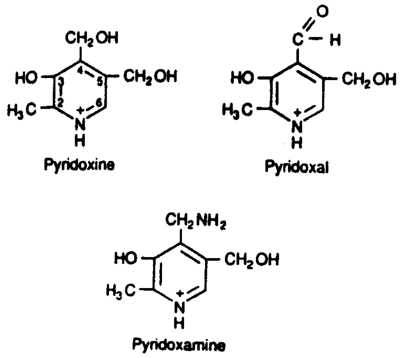
Deficiency :

Deficiency is rare because pantothenic acid is widely distributed in food and readily absorbed in the intestine and subsequently transformed to COASH & CAP.

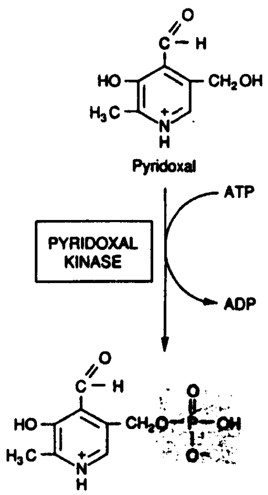
VITAMIN B_6

Chemistry

- Vit. B_6 consists of three closely related **pyridine** derivatives namely pyridoxine, pyridoxal & pyridoxamine.
- The biologically **active forms** are the **phosphates of pyridoxine, pyridoxal and pyridoxamine**. (pyridoxal phosphate is the major form in the plasma of humans). Phosphorylation is catalyzed by pyridoxal kinase using ATP.



Naturally occurring forms of vitamin B_6.



The phosphorylation of pyridoxal by pyridoxal kinase to form pyridoxal phosphate.

Function :

Pyridoxal phosphate (PP) and pyridoxamine phosphate act as coenzymes involved in amino acid and glycogen metabolism :

1) Transaminases : It is the transfer of an amino group from an α-amino acid to an α-keto acid, forming new α-kito acid and α-amino acid, respectively. Pyridoxal phosphate, First is combined to amino group of α- amino acid forming pyridoxamine phosphate. Second : PP is released again at the end of transamination.

2) Decarboxylases : It is the prosthetic goup of amino acid decarboxylases and transform amino acids into the respective biologically active amines like :

   - Histidine → Histamine + CO_2

   - Tyrosine → Tyramine + CO_2

   - Tryptophan → Tryptamine + CO_2

   - Glutamate → Gama Amino Butyric Acid (GABA) + CO_2

3) Glycogen phosphorylase : It forms an integral part of liver & muscle glycogen phosphorylases which degrades the 1,4 glycosidic linkage of glyeogen to form glucose -1-phosphate.

Deficiency :

Vit B6 deficiency alone is rare and only occurs during multiple Vit B-complex deficiencies. Therefor it occurs in :

1) Infants whose mothers are depleted of the vitamin after being kept on diet poor in this vitamin.

2) Tuberculous patients taking the drug **isoniazide** by forming inactive derivative between the drug & pyridoxal.

3) Alcoholics who metabolize ethanol into acetaldehyde which stimulates hydrolysis of the phosphateof the coenzyme PP.

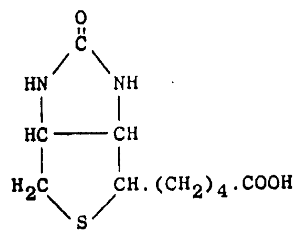
Manifestations of deficiency include :

1) Convulsions in infants due to deficiency of GABA which is a neurotransmitter
2) Hypochromic anaemia due decreased heme synthesis.
3) Loss of appetite, nausia, vomiting & glossitis.
4) Pellagra like symptoms due to decreased niacin synthesis from tryptophan (Refer to niacin).

BIOTIN

Chemistry :

Consists of **two fused rings, imidazole & thiophene**, with valeric acid side chain linked to thiophene ring.



Biotin

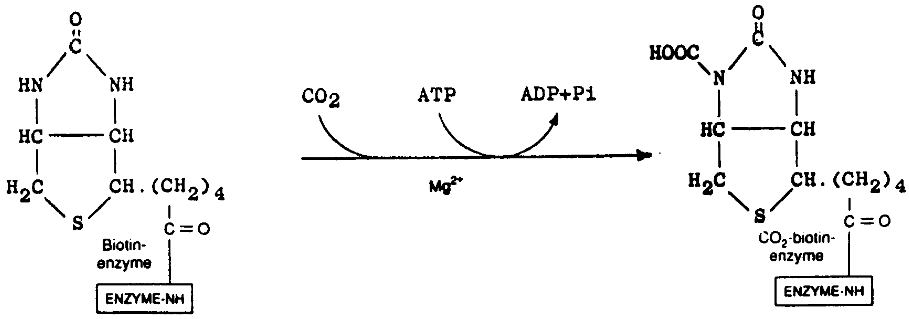
Function :

- Biotin is the prospthetic group of enzymes involved in **carbon dioxide fixation** or carboxylation reactions **(carboxylases)**.

A) CO_2 fixation occurs in two steps :

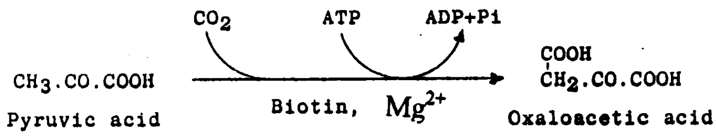
1) CO_2 is attached to N_1 of biotin generating an activated intermediate (carboxy- biotin enzyme) in the presence of ATP, HCO_3^- and Mg^{2+}.

2) The activated carboxyl group is then trnasferred to the substrate of the reaction.



B) Carboxylation reactions include :

1) Pyruvate carboxylase : In citric acid cycle.



2) Carbamoyl phosphate synthetase : In urea cycle and pyrimidine synthesis :

$CO_2 + NH_3 + ATP \rightarrow$ Carbamoyl phosphate.

3) Acetyl - COA carboxylase : In fatty acid synthesis

CO_2 + acetyl COA +ATP $\rightarrow$ malonyl COA + ADP + Pi.

4) Fixation of CO_2 at C_6 of purines : In purine synthesis

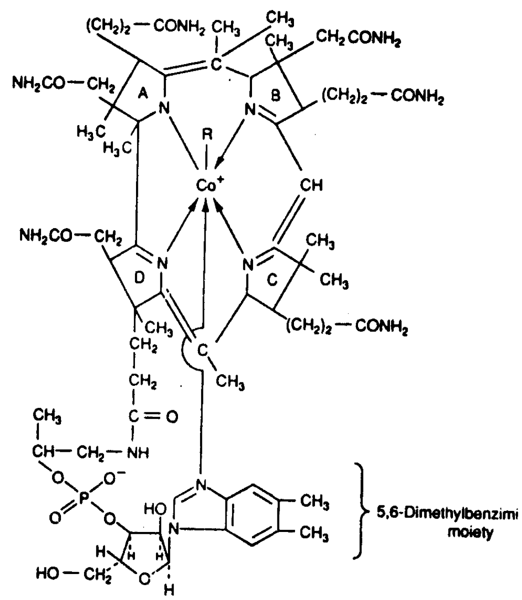
Deficiency :

- It is very rare in humans.
- Induced by ingestion of large amount of raw egg white which contains the heat labile **avidin** that combines very tightly with biotin preventing its absorption from intestines → deficiency.
- Symptoms include : Dermatitis & muscle pain, hallucination, depression, loss of hair, loss of appetite anaemia.
- Symptoms are due to accumulation of substrates of biotin- dependant enzymes that appear in urine. These metabolites include : lactate, β-hydroxy propionate and β- hydroxy isovalerate.

VITAMIN B_{12} (Copalamine)

Chemistry :

Complex structure **(corrin ring)** similar to a porphyrin ring (4 pyrol rings) to which **cobalt ion** (trivalent) is added to its center. Cyanocobalmine is formed when a cyanide group is added to the cobalt.



Vitamin B_{12} (cobalamin). R may be varied to give the various forms of the vitamin, eg, R = CN in cyanocobalamin; R = OH in hydroxo- cobalamin; R = 5′ -deoxyadenosyl in 5′-deoxy- adenosylcobalamin; and R = CH_3 in methyl- cobalamin.

Synthesis :

Only by microorganisms & stored in animal tissues, mainly in the liver as methyl-, adenosyl- & hydroxycobalamine. It is absent from plants.

Absorption :

Vit B_{12} → intestine → binds to a mucoprotein named **"intrinsic factor"** → complex → binds to a receptor on intestinal cells → transported to portal blood → liver → intrinsic factor is released again → binds another Vit B_{12} molecule.

Transport in blood :

Bound to β-globulin (transcobalamine II) or α-globulin (transcobalamine I) for transport to tissues & storage in the liver.

Active forms :

1) Hydroxocobalamine : in the cytosol of cells.
2) Methylcobalamine : in the cytosol of cells.
3) 5 - deoxyadenosyl cobalamide **(cobamide)** in the mitochondria (it has an orange color).

Functions :

A) Cobamide is the coenzyme for the enzyme methylmalonyl isomerase :

Methylmalonyl COA → **succinyl** COA → Krebs cycle → gluconeogenesis.

B) Methylcobalamine is the coenzyme for transmethylation :

1. Methionine synthesis in animal tissues:

Homocysteine + methylcobalamine → methionine + cobalamine → + methyltetrahydrofolate → tetrahydrofolate + methylcobalamine → + homocysteine → methionine + cobalamine.

2. Thymine synthesis in nucleic acids :

methylcobalamine + d-uridine monophosphate → d- Thymidine phosphate → DNA synthesis.

Deficiency :

A) Deficiency of intrinsic factor (after gastrectomy) → prevents Vit B_{12} absorption from intestine.

B) Vegetarians (those who eat vegetables only) are at risk of developing Vit B_{12} deficiency.

C) There is impaired methionine & nucleic acid synthesis preventing cell divisions and formation of big red blood cells **(megalobalasts)** in the bone marrow and **megaloblatic anaemia**.

D) Tetrahydrofolate deficiency and excess methyltetrahydrofolate **(Folate trap)**.

E) Homocystinuria & methylmalonic aciduria.

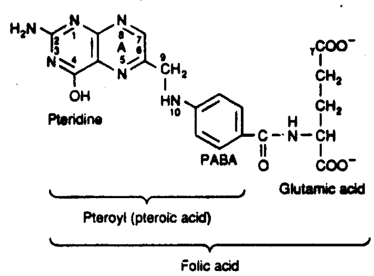
F) Neurological manifestations : peripheral neuritis and **subacute combined degeneration of the spinal cord**.

G) **Pernicious anaemia** (megaloblastic).

FOLIC ACID

Chemistry :

It is pteroylglutamic acid (pteridine nucleus + paraminobenzoic acid (PABA) + glutamic acid).

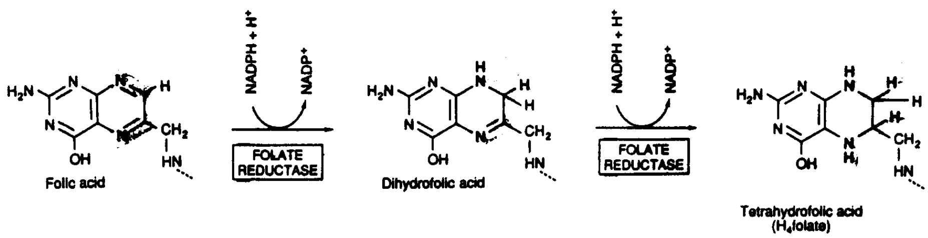


Synthesis :

By microorganisms and plants but not animals or humans. In plants it is present as pteroylheptaglutamate containing polyglutamate conjugate consisting of δ-linked polypeptide chain of 7 glutamate residues. Poly-glutamates are hydrolyzed in the intestine & liver to pteroylmonoglutamate & glutamic acid.

Active forms :

It is tetrahydrofolate (H_4 folate or FH_4) prdouced after reduction of folic by folate reductase enzyme using $NADPH+H^+$ as coenzyme.



Methotrexate (anticancer drug) inhibits the function of folic acid by competitive inhibition of folate reductase enzyme.

Functions :

A) FH_4 serves as a carrier of one-carbon units (**C_1 unit**) like methyl **($-CH_3$)**, methylene **($-CH_2-$)**, methenyl **(= CH-)**, formyl **(- CHO)** and formimino **(-CHNH)**; they are interconvertible.

B) Sources of one-carbon unit :

1) Serine : The main source (Its β-carbon).

- Methylation of homocysteine to methionine using cobalamine as a cofactor.

Serine + FH_4 $\rightleftharpoons$ Glycine + N^5 N^{10} methylene FH_4

$\xrightarrow{Reduced}$ N^5 methyl FH_4 $\xrightarrow{Cobalamine}$ methylcobalamine

$\xrightarrow{Homocysteine}$ methionine + cobalamine

- N^5 N^{10} methylene FH_4 is oxidized to N^5 N^{10}- methyl FH_4 then hydrated to N^5 formyl FH_4.

2) Glycine : Its α-carbon is the source.

- Cleavage of glycine to CO_2, NH_4^+ & N^5 N^{10}. methylene FH_4.

Glycine + NAD + FH_4 $\xrightleftharpoons{\text{Glycine Synthase}}$

CO_2 + NH_4^+ + NADH + H + N^5 N^{10}. metheyleneFH_4.

3) Tryptophan :

- Tryptophan $\longrightarrow$ formic acid $\xrightarrow{FH_4}$ N^{10} - formyl FH_4

4) Histidine :

- Histidine $\longrightarrow$ formiminoglutamate (Figlu).

$\longrightarrow$ + FH_4 $\longrightarrow$ N^5. formimino FH_4.

C) Fate of one – carbon unit :

1) Serine synthesis :

Glycine + N^5 N^{10} methylene FH_4 $\longrightarrow$ serine + FH_4.

2) Methionine synthesis :

3) Thymine synthesis (DNA synthesis)

Uridine monophosphate + N^5 N^{10} methylene FH4 $\longrightarrow$ Thymidine monophosphate + FH_2.

4) Purine synthesis (DNA & RNA synthesis) N^5 N^{10} methenyl FH_4 form C_8 of purine ring.

D) Nucleic acid synthesis :

FH_4 is important for thymine & purine synthesis and therefor is important for hemopoisis.

Deficiency :

A) Megaloblastic anaemia : Due to defective DNA synthesis and enythrocyte formation. Megaloblastic anaemia, caused by Vit B_{12} deficiency, may be treated by giving more folic acid in the diet because both Vit B_{12} and Folic acid participate in methionine synthesis.

However folic acid will not cure the neurological manifestations of vit B_{12} deficiency.

B)Impaired growth :

Diagnosis of folic acid deficiency :

By **figlu test** : increased urinary excretion of formininoglutamic acid **(figlu)** after a big dose of histidine.

VITAMIN C (Ascorbic acid)

Chemistry :

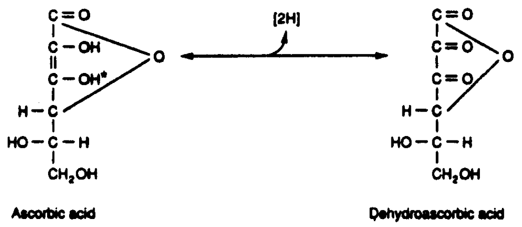
L-ascorbic acid. It is derived from glucose.

Sources :

Fresh vegetables, fruits (citrus fruits, guava) & germinating seeds.

Properties :

1) Stronger acid than acetic acid.



Ascorbic acid, and its oxidation to dehydroascorbic acid. *(ionizes in ascorbate).

2) Stable in acid but unstable in alkalies.

3) Unstable to cooking and canning of food.

4) Metabolized in humans to oxalic acid → urine.

Active forms :

L-ascorbic & dehydroascorbic acids (D-form is inactive).

Functions :

1) It is very sensitive to reversible oxidation(L-ascorbic acid ⇄ L-dehydroascorbic acid).

2) It is a strong reducing agent.

3) It should be preserved in the reduced form (L-ascorbic acid) by sulfhydryl compounds like glutathione & cysteine.

4) It can reduce molecular oxygen, nitrate & cytochromes a & c.

5) It is a reducing cofactor in several enzymatic reactions :

   a. Proline & lysine hydroxylases: in collagen synthesis in fibrobasts (Fe^{2+} is a metal cofactor).

   b. Mono & dioxygenases : ascorbic acid keeps their metal cofactors (Cu^{2+} & Fe^{2+}) in the reduced form.

   c. Dopamine hydroxylase : in adrenaline synthesis from tyrosine.

   d. P-hydroxyphenylpyruvate hydroxylase : in tyrosine degradation (Cu^{2+} is a metal cofactor).

   e. Steroid hormone synthesis in adrenal cortex.

6) Keeps dietary iron in ferrous form & help its intestinal absorption & its mobilization from its stores.

7) Acts as a general water soluble antioxidant & inhibits nitrosamines during digestion.

8) Important for the formation of chondroitin sulfate of connective tissues & cement substances of capillaries.

Deficiency :

Vit C deficiency causes a disease called Scurvy.

1) There is **defective collagen & connective tissue synthesis** leading to :
   - Delayed wound healing, bone formation & teeth eruption.
   - Muscle weakness, soft swollen gums & loose teeth.

2) Decreased iron absorption leads to **anaemia**.

3) Weak blood capillaries leads to **subcutaneous haemorrhages (petichae)**.

FAT SOLUBLE VITAMINS

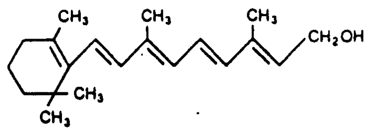
Common properties :

1) Lipid soluble (apolar hydrophobic molecules) which are all **isoprene derivatives**.
2) Not synthesized in the body & must be supplied in diet.
3) Absorbed with fat in the intestine.
4) Transported in blood with lipoproteins or attached to specific binding protein.

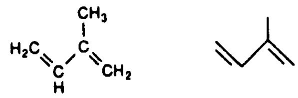
VITAMIN A

Chemistry & properties :

1) Vit A **(or retinol)** is a polyisoprenoid compound containing a cyclohexenyl ring.



Retinol (vitamin A).



Two representations of isoprene.

2) It is a derivative of carotenoids which contain a **β-ionone ring** like α-, β- & β carotenes (provitamine A).

3) Vit A_1 (retinal) : formed of β-ionone ring + 2 isoprene units + hydroxymethyl group.

4) Vit A_2 : contains extra double bond in the β-ionone ring.

5) Vit A_1 (retinal) in the intestine is :

a. Reduced to retinol (Vit A) using NADPH $+H^+$.

b. Oxidized to retinoic acid using NAD.

6) **Retinol, retinal** and **retinoic acid** all have Vit A activity.

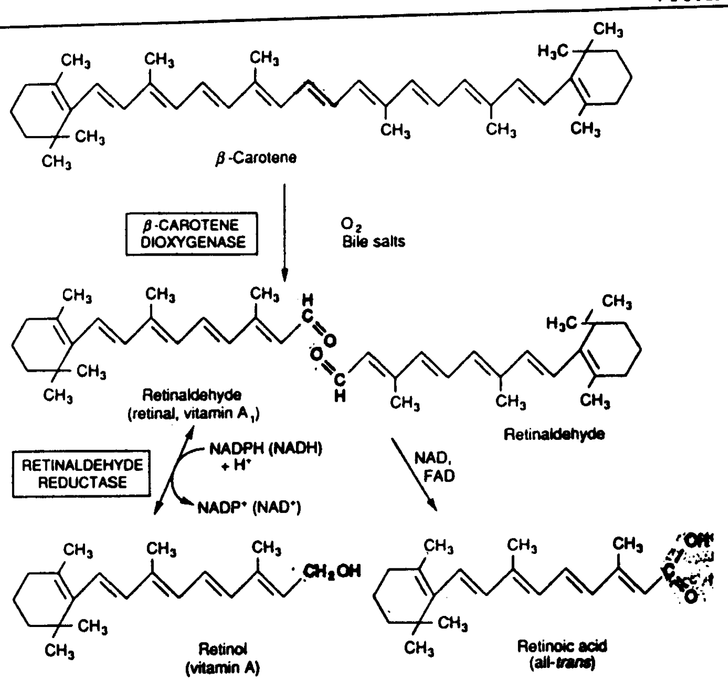
7) Retinol is estrified with saturated fatty acids in the intestine then it is absorbed and stored in the liver as a **lipoglycoprotein complex**.

8) It is released from the liver by hydrolysis then it is bound to aporetinol **binding protein (RBP)** & transported to tissues where it is taken up into them through cell surface receptors.

9) Once inside cells it is bound to **cellular retinol binding protein (CRBP)**.

Sources :

1) **Cartotenoids** (provitaminA) occur in both plant and animal tissues. Carotenoids of animals are derived from plants. Plant sources include yellow-red colored vegetables & fruits like corn, sweat potatoes, carrots, tomatoes, apricot, yellow peaches... etc.

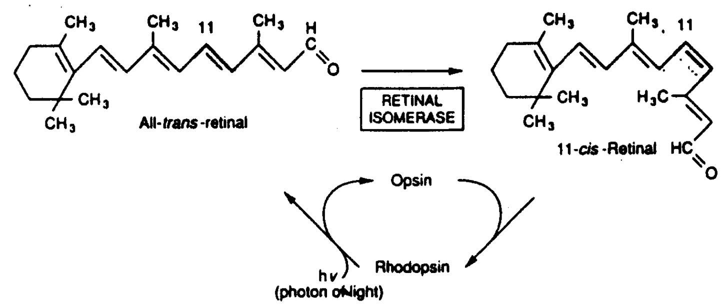


2) Vit A occurs only in animal tissues like whole milk, butter, egg yolk... etc. The liver of certain fish contains higher concentration of Vit A than do any other tissues (marine fishes have Vit A_1 while fresh water fishes have Vit A_2) (halibut liver oil, cod liver oil, tuna liver oil).

Function :

1) **Vision (Retinal) :**

- Retina of the eye contains two types of **receptors** : **cones** (for color vision and vision in bright light) and **rods** (for night vision).
- Night vision depends on vitamin A content in rods.
- The vitamin is present in rods as retinal **(Vit A_1)** which forms a photoasensitive pigment, **rhodopsin** or visual purple.
- **Rhodopsin** is formed of a protein, **opsin**, combined with **11-cis-retinal** (all trans-retinal cannot combine with opsin).



11-*cis*-Retinal, formed from all-*trans*-retinal, combines with opsin to form rhodopsin in the rod cell of the eye. The absorption of a photon of light by rhodopsin causes it to bleach, generating opsin and all-*trans*-retinal. Retinal is require to maintain this cycle of reactions.

- Exposure to light : the double bond in retinal will change from Cis- to all - trans and **all-trans – retinal** will dissociate from opsin and rhodopsin is converted to a colorless pigment. These photochemical changes areaccompanied by conformational changes in rod cell membrane that induces **a calcium ion channel** in them → rapid influx of calcium into rod cells → nerve impulse → light is perceived by the brain.
- **Regeneration of rhodopsin** occurs by transforming all-trans-retinal into 11-cis retinal by **retinal isomerase** enzyme in the **liver**.
- In the **dark**, good vision depends on the rate of generation of rhodopsin in the retina after combining 11-cis retinal present in blood with opsin present in rods.

2) Glycoprotein synthesis (retinoic acid) :

Retinoic acid in the form of retinoyl phosphate carry oligosaccharides across cell membranes (retinoic acid is lipophilic) allowing them to combine with proteins. Therefor Vit A is important for normal growth.

3) Proteoglycan synthesis :

Vit A is important for chondroitin sulfate synthesis in bones & teeth.

4) **Reproduction (retinal) :**

Retinal controls the **expression of certain genes** for reproduction.This occurs by binding retinal, carried byCRBP (cellular retinal binding protein), to nuclear proteins inducing changes in gene expression affecting reproduction. In this respect they **behave – like steroid hormones**.

Deficiency :

1) Defective night vision **(night blindness)**.
2) Other changes in the eye (severe deficiency) : Xerophthalmia & keratinization of epithelial tissues of the eye (cornea) → blindness.
3) Keratinization of epithelial tissues of the lung, gastrointestinal tract, genitourinary tract & skin.
4) Defective bone & teeth growth.

Vit A hypervitaminosis :

1) Follow ingestion of very large amounts of Vit A as in :
   a) Eskimos who ingest large amount of polar bear livers.
   b) Children who continuously take excessive amount of the vitamin leading to :
      - Roughening of skin, irritability, falling of hair, loss of weight, hyperlipaemia & haemorrhages.
      - Acceleration of bone growth & early closure of epihyses & retardation of growth.

Therepeutic use :

1) Anticancer : Vitamin A administration decreases the risk of cancer due to stimulation of the immune system.

2) Antioxidant : Vit A removes free radicals in tissues. This effect when combined with the antioxidant effect of Vit E will increase their auticancer activity.

VITAMIN D

Chemistry & Synthesis :

A) Vitamins D_2 & D_3 are **steroid prohormones.**

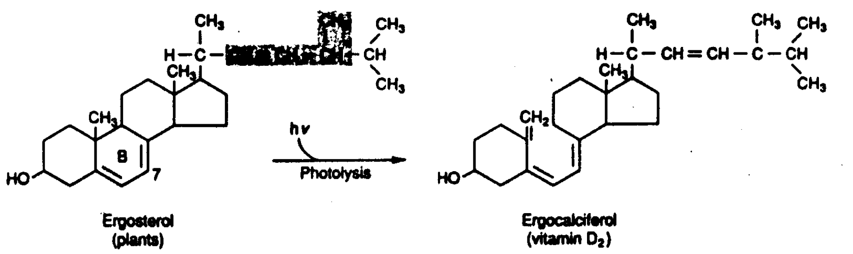
1. Ergosterol **(in plants)** is provitamin D_2 :

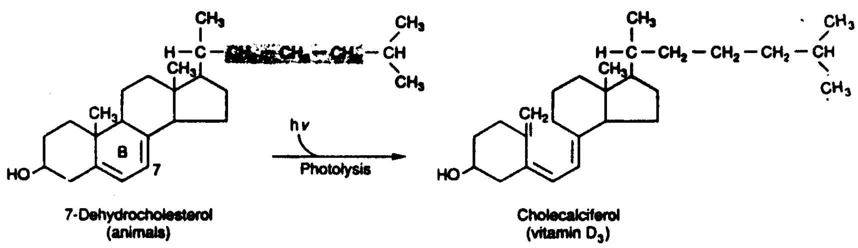
   Ergosterol + sun light → Vit D_2 (Ergocalceferol)

2. 7-dehydrocholesterol **(in animals)** is provitamin D_3 :

   3-dehydrocholesterol + sun light → Vit D_3 (Cholecalicferol).

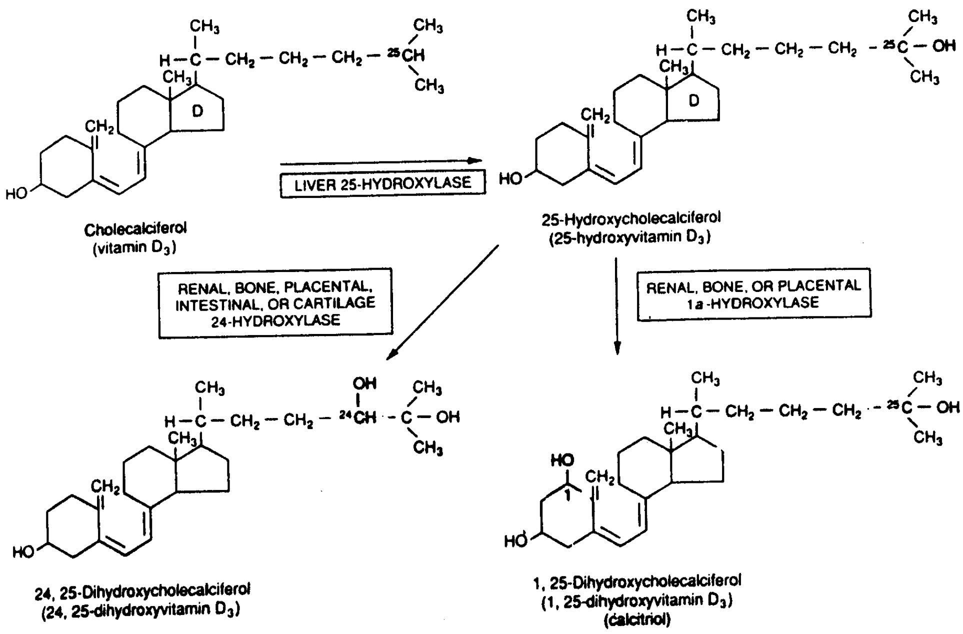
B) Ultraviolet light (or sun light) cleaves the B ring of both provitamins D_2 & D_3. giving Vit D_2 & D_3 prohorrmones (ergocalciferol & cholecalciferol, respectively).





C) Synthesis of **calcitriol**, (1,25 – dihydroxy D_3) :

1. It is the **active form of Vit D_3.**
2. Synthesis occurs in **two stages** :
   a. In the liver Vit D_3 is hydroxylated at 25 position by Vit D3 – 25 - hydroxylase → **25-hydroxy D_3** → blood → stored in the liver cytosol.
   b. In the renal tubules, bone & placenta the 25-hydroxy Vit. D_3 is further hydroxylated in 1 position by 25-hydroxy D_3 1 α-hydroxylase in the mitochondria → **1,25 – dihydroxy D_3 (calcitriol).**



Sources :

1) Ergosterol : In yeast (the most abundant source), animal tissues (from plant source) like chicken egg, snails & milk. It is not absorbed from the nitestine & therefor it is of no nutritional value.

2) 7-dyhydrocholesterol : in animals like egg yolk, cow milk, human milk, fish liver (the highest content in cod liver oil, halibut – liver oil & tuna liver oil). It is synthesized in the intestine from cholesterol and passes to the skin where it undergoes activation to Vit D_3 (cholecalciferol) by solar rays. Then, Vit D_3 is bound to specific transport protein **(D – binding protein)** which moves D3 from skin & intestine to the liver for 25-hydroxylation step.

Functions of calcitriol :

1) Increases intestinal absorption of calcium and phosphorus against concentration gradient.

2) Increases renal tubular reabsorption of calcium & decreases excretion of phosphate in urine.

3) Both 1 & 2 increase calcium and phosphate levels in blood helping their deposition as **hydroxyapatite crystals** on to the **collagen fibers of bones** → increased calcification of bone. The producton of 1, 25 – dihydroxy D_3 by the kidney is inhibited by high blood calcium levels due to feed back inhibition of 1-hydroxylase enzyme.

Mechanism of action of calcitriol :

- Similar to **steroid hormone** action on cellular level.
- Calcitriol binds to **calcitriol receptor** in the cytosol (member of steroid hormone family) in specific, saturable and reversible way.
- Vit D_3 + receptor complex binds to DNA which stimulates gene transcription → specific m RNA → protein biosynthesis of **calcium binding protein** (CBP) by intestinal mucosal cells which binds calcium and helps transport of it across the brush border of these cells. Phosphate absorption is secondary to calcium absorption.

Deficiency :

1) Vit D deficiency leads to defective intestinal absorption & renal reabsorption of both Ca & phosphorus → low Ca & ph. levels → increased parathyroid hormone activity → demineralization of bone to maintain blood calcium level → later on, **severe decrease of blood calcium and phosphate levels** occur → **soft bones.**

2) **Rickets** or bone softening in children due to decreased calcium & phosphorus in bones. The main manifestations include :

- Large & soft head with delayed closure of fontanelles.
- Enlarged ends of long bones (tibia & radius) & lower limbs bend outwards or inwards
- Deformed chest.

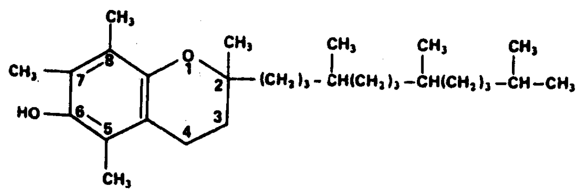
3) **Osteomalacia** or bone softening in adults where bones get easily fractured.

VITMAIN E (Tocopherols)

Chemistry & Properties :

1) Naturally occuring tocopherols are isoprenoid substitued 6-hydroxychromanes or tocols- (α, β, γ and δ tocopherols). D α-tocopherol has the widest natural distribution & is the most active (5,7, 8 – trimethyltocol). Tocol contains the **chromane nucleus.**

2) Stable to heat, soluble in fat, insoluble in water & destroyed by light & O_2.



α-Tocopherol.

Sources :

1) Plants : Wheat germ oil is the richest source. Green leafy vegetables are good source.

2) Animals : liver, egg yolk & milk.

Absorption & transport :

1) First it is liberated from lipids then absorbed during fat digestion.

2) Then it is transported in the blood by lipoproteins → chylomicrons → tissues containing lipoprotein lipase → liver → incorporated in VLDL → stored in adipose tissues.

3) Deficiency occures in conditions associated with dysfunction of absorption & transport of lipids like chronic steatorrhoea, cholestatic liver diseases, abetalipoproteinemia & in patients who undergone intestinal resection.

Function :

1) **The most potent antioxidant**. It prevents the toxic effect of peroxidation of polyunsaturated fatty acids **(PUFA)** present in the phospholipid part of membranes. Therefor Vit E prevents destruction of cell membranes.

2) Free radicals :

- Free radical is an atom or molecule that has one or more unpaired electrons which were acquired from other substances.
- Free radicals are highly reactive & potentially damaging to tissues (membranes, enzymes, nucleic acids).
- Free radicals include : **superoxide, hydrogen peroxide & hydroxyl** (hydroxy free radical is the most toxic & short lived).

- Free radicals are formed during certain metabolic processes such as **xanthine oxidase, lipoxygenase, cyclooxygenase reactions** which produce superoxides (O_2^{-}), peroxyl ($ROO^{\bullet}$) and hydroxyl ($OH^{\bullet}$) radicals, respectively. **Superoxides** may also be produced during xenobiotics by **cytochrome P-450**.

3) Vitamin E prevents free radical formation through its **ability to transfer a phenolic hydrogen to peroxyl free radical ($ROO^{\bullet}$)** of peroxidized polyunsaturated fatty acids (PUFA).

$$ROO^{\bullet} + Toc\ OH \longrightarrow ROOH + Toc\ O^{\bullet}$$

4) Toc OH is regenerated from Toc $O^{\bullet}$ by :

a) Vit C (reduced) + Toc $O^{\bullet}$ $\rightarrow$ Vit C (oxid) + Toc OH.

b) Glutathione (G-SH) + Toc $O^{\bullet}$ $\rightarrow$ G S-SG + Toc OH.

5) The antioxidant effect of Vit E prevents certain diseases like cancer and cardiovascular diseases.

6) Vit E deficiency leads to anaemia in newborn infants due to decreased hemoglobin synthesis & short red blood cell life span.

7) Increased intake of PUFA increases the requirement of Vit E in the diet.

VITAMIN K

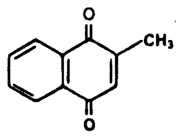
Chemistry :

Polyisoprenoid substituted **naphthoquinones. Vitamin k series include**:

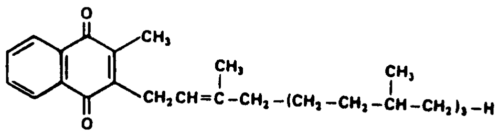
1) Vit K_3 (Menadione) : It is **synthetic** 2-methyl-1, 4-naphthoquinone. It is the parent compound of the naturally occuring Vit K_1 & K_2.

2) Vit K_1 (Phylloquinone) : Produced by **plants and has a phytyl chain** attached to menadione (k_3).

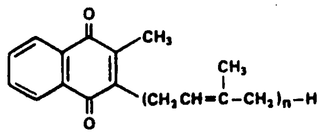
3) Vit K_2 (Menaquinone) : Produced by **bacteria** and has a difarnesyl chain attached to menadione (K_3).



Menadione (vitamin K_3)



Phylloquinone (vitamin K_1, phytonadione, Mephyton)



Menaquinone-n (vitamin K_2; n = 6, 7, or 9)

<u>Sources :</u>

1) Vit K_1 : Present in plants (originally isolated **from alfalfa**). Good source is green leafy vegetables, cabbage, spinach, tomatoes, cauliflower ... etc.)

2) Vit K_2 : Produced by intestinal bacteria. Vit K_2 found in animal tissues is of bacterial and plants origin (milk, egg yolk, liver... etc).

<u>Properties :</u>

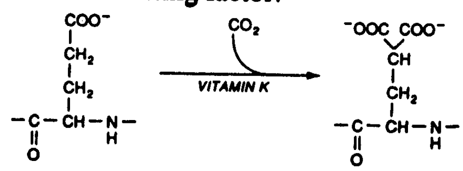
3) Vit K_1 & K_2 are fat soluble while Vit K_3 (synthetic) is soluble in water and is easily absorbed even in the absence of bile or other lipids.

4) Heat stable & destroyed by light.

Function :

1) Synthesis of blood clotting factors : Vit K is involved in the synthesis of blood clotting factors II, VII, IX and X by the liver. This occurs through activation of a precursor.

- The **hydroquinone** form of Vit K acts as a **coenzyme** for **carboxylase** enzyme which induces posttranslational modification of **glutamate** residue (Glu) of **precursor proteins of blood clotting factors** (e.g. factor II or prothrombin) to **δ-carboxylglutamate**. The new carboxyl binds calcium and thus allow **calcium chelation** which is essential for the biological activity of the blood clotting factor.



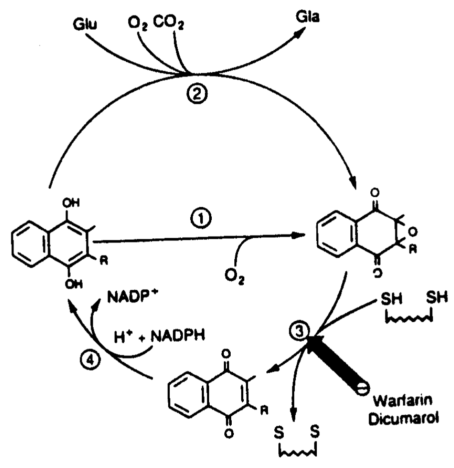
Carboxylation of a glutamate residue catalyzed by vitamin K-dependent carboxylase.

2) **Vit K cycle** : It is the cycle by which the active reduced form of Vit K **(hydroquinone)** is **regenerated** back at the end of carboxylation of glutamate residue of precursors of blood clotting proteins.

Protein – **Glu** + hydroquinone (Vit K) —($CO_2 + O_2$)→ Protein-**carboxy- Glu**

Vit K – 2,3 – epoxide → Quinone (Vit K) → hydroquinone (Vit K)

3) **Dicumarol** (Warfarin) : It is an **anticoagulant drug,** being Vit K antagonist. It acts by inhibiting the step of transformation of Vit K epoxide into quinone form of Vit K during Vit K cycle.



① monoxygenase; ② carboxylase; ③ 2,3-ep- oxide reductase; ④ reductase.

Deficiency : It is rare in adults.

1) Causes :

a) Excessive intake of antibiotics inhibit Vit K synthesis by intestinal bacteria.

b) In newborn infants because the placenta does not allow the passage of maternal Vit K to the fetus & due to absence of bacteria in their intestine.

c) Fat malabsorption associated with pancreatic dysfunction, biliary diseases & steatorrhoea.

2) Effect : Vit K deficiency causes **hemorrhagic diseases** due to decreased synthesis of blood clotting factors (mainly prothrombin). External & internal bleeding accidents might occur.

DIGESTION

Definition :

Digestion is the process of breakdown of naturally occurring foodstuffs (polymers) into smaller molecules (monomers) before they can be absorbed then metabolized by all cells of the body.

General Considerations :

A) Sites of digestion :

1) Intraluminal : in the stomach (minor part) and the intestinal (major part) lumen. Digestion occurs by gastric, pancreatic & biliary juices.
2) Brush border of enterocytes (epithelial cells that line the lumen of the small intestine) : produce enzymes that digest oligomers and dimers that result from pancreatic digestion.
3) Intracellular digestion : hydrolysis of di-& tripeptides in the cytoplasm of enterocytes.

B) Proenzyme form of digestive enzymes :

Digestive enzymes are synthesized in specialized cells (salivary gland, stomach & pancreas) then stored in secretory vesicles (zymogen granules) as inactive **proenzymes or zymogens**. These granules move and fuse with plasma membrane and their contents are released into the lumen by exocytosis.

C) Secretagogues regulate enzyme secretion :

Secretagogues are substances that interact with receptors on the surface of exocrine cells. This will initiate certain signals ending by fusion of zymogen granules with the plasma membranes & stimulation of digestive enzyme secretion.

These signals are **similar to those involved in hormone action** ending by either Ca^{2+} release into the cytosol or production of cyclic AMP (these are **second messengers** in hormone action).

Examples of secretagogues :

1) Acetylcholine : stimulates secretion of salivary amylase, HCl, pepsinogen trypsinogen, chymotrypsinogen & Nacl of salivary gland & intestine.
2) Secretin hormone from small intestine : stimulates secretion of pancreatic juice rich in Nacl & NaH CO_3.
3) Cholecystokinin (pancreozymin) hormone from small intestine : stimulates secretion of pancreatic juice & contraction of the gall bladder.
4) Gastrin hormone from stomach : stimulates gastric Hcl & pepsin secretion
5) Biogenic amines :
   a) Histamine from stomach : it interacts with gastric - specific histamine receptor (H_2 receptor) on parietal cells of stomach inducing HCl secretion.
   b) Serotonine (tryptophan metabolite) is present in high amounts in the gastrointestinal tract. It stimulates intestinal Nacl secretion.

Digestion in the oral cavity :

A) Saliva : it is a colorless viscous fluid present in the mouth (oral cavity), secreted by salivary glands (parotid, submandibular & sublingual) and acts as lubricant for mastication.

1) Volume = about 1.5 litres / day, pH = 6.4 - 6.8.
2) Composition :
   a) Water = 99.4%

b) Solids = 0.6 % and include :

- Inorganic substances (0.2 %) : Na^{+}, Cl^{-}, HCO_3^{-} and little K^{+} & HPO_4^{2-}.
- Organic substances (0.4%) : include mucin (glycoprotein which gives saliva its viscosity) and salivary amylase **(ptyalin)**.

B) Salivary amylase (ptyalin) :

1) Optimum pH = 7.0, activated by Cl^{-} ions & act in the mouth.
2) Activity stops at pH below 4.5 (in the stomach).
3) It is an endosaccharidase (α-amylase) that attacks α-1,4-glucosidic bonds in the middle of branches of starch & glycogen (it does not attack α-1,6 bonds at the branch points).
4) Steps of digestion of starch or glycogen :

Starch ⟶ amylodextrin ⟶
(↘ Maltose) (↘ Maltose)

Erythrodextrin ⟶ Achrodextrin ⟶ Maltose
(↘ Maltose)

5) Complete digestion of polysaccharides in the mouth is rare and it depends on the rate of mastication of food in the mouth.

Gastric digestion :

A) Gastric juice :

1) Volume = about 2.5 litres / day, pH = about 1.5 in adults (infants have lass acidic juice, pH = about 5.0).
2) Composition :

a) Water = 99.4 %

b) Solids = 0.6 % and include :

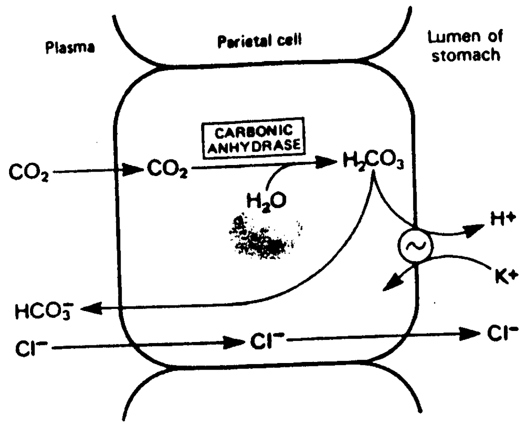
- Inorganic substances (0.2%) : Hcl, Na^+ and little K^+ & HPO_4^{2-}
- Organic substances (0.4%) : include mucin, intrinsic factor (for Vit B_{12} absorption) and enzymes (pepsin, Lipase & renin).

B) Gastric HCl :

1) Secreted by the **parietal (oxyntic) cells** : of gastric glands.

2) Mechanism of secretion of HCl.

Production of gastric hydrochloric acid. ⊖ H^+/K^+-ATPase.



a) Source of H^+ of HCl :

- H_2CO_3 is formed in parietal cells by **carbonic anhydrase** catalyzed reaction after passage of CO_2 from plasma to cells :

$$CO_2 + H_2O \xrightarrow{\text{Carbonic anhydrase}} H_2CO_3$$

- H_2CO_3 dissociates into H^+ & HCO_3^- inside parietal cells.
- **Then H^+** is secreted into the stomach lumen by an active process that involves **K^+, H^+. ATPase enzyme** located in the membrane of cells. K^+ passes from the lumen of stomach to inside of cells in exchange for H^+ using energy

released from hydrolysis of ATP by K^+, H^+. ATPase enzyme.

b) Source of Cl^- of HCl :

- Cl^- ions pass from blood to the inside of parietal cells.
- Then Cl^- ions pass to the lumen of stomach in exchange for HCO_3^- that passes from cells to blood plasma.
- This Cl^- & HCO_3^- exchange is **similar** to the **chloride shift** that occurs in red blood cells.

c) Alkaline tide :

- It explains why urine becomes alkaline after ingestion of a meal.
- It is due to formation of HCO_3^- in the parietal cells → NaHCO3 (alkaline) → blood → urine during the process of Hcl secretion after a meal.

3) Function of HCl :

a) Denaturates proteins making them more affected by proteolytic enzymes.

b) Kills bacteria due to low pH of stomach.

c) Activates pepsinogen → pepsin.

d) Provides optimum pH for action of pepsin.

e) Increases Ca^{2+} absorption by converting insoluble calcium salts into soluble calcium chloride.

f) Increases iron absorption by converting insoluble organic ferric compounds to soluble ferric chloride.

C) Gastric digestive enzymes :

1) Pepsin

a) Optimum pH : 1.5 - 2.5

b) Secreted by **chief cells** of gastric glands as inactive pepsinogen.

c) Pepsinogen is activated to pepsin by removal of 44 amino acids from the N-terminal by two ways : i) H^+ of Hcl ii) by pepsin itself (autocatalytically).

d) It is an **endopeptidase (protease)** that hydrolyzes peptide bonds within the polypeptide structure.

e) It is specific for peptide bonds formed by : a) Aromatic amino acids (Phe, Tyr) b) Dicarboxylic amino acids (Glu, Asp).

f) Products of digestion are **peptide fragments** (proteoses & peptones) & some free amino acids.

2) Gastric lipase :

a) Optimum pH = 6.0

b) Inactive in adults due to low stomach pH (1.5 - 2.5).

c) Active in stomach of infants (pH = 6.5) & digest milk fat.

d) Similar in action to pancreatice lipase.

e) It has particular importance in neonatal life when pancreatic lipase is deficient.

3) Rennin :

a) Optimum pH = 5.0

b) Absent from human stomach.

c) Inactive in adults due to low stomach pH.

d) Active in **infants** : It coagulates milk and prevents its rapid passage from the stomach to intestine.

e) Milk clotting :

$$\text{Casein (milk protein)} \xrightarrow{\text{Rennin}} \text{soluble parcasein}$$

$\xrightarrow{Ca^{+2}}$ calcium paracaseinate (milk clot or curd).

f) Pepsin then digests milk clot.

g) Rennin is used for making cheese.

Intestinal digestion :

Stomach contents **(chyme)** pass to the duodenum and its acidic nature is neutralized by the alkaline contents of the pancreatic & biliary secretion followed by change of its pH to alkaline side. This alkalinity is necessary for the activity of the enzymes of pancreatic & intestinal juice.

A) Pancreatic juice

1) Volume = about 700 ml/ day, pH = about 8.0 due to high bicarbonate content.

2) Composition :

a) Water = 98.4 %

b) Solids = 1.6 % and include :

- Inorganic substances (1.0%) : Na^+, HCO_3^- , K^+, Cl^- and little Ca^{2+}, HPO_4^{2-} & Zn^{2+}.
- Organic = 0.6 % are enzymes

B) Pancreatic enzymes :

1) Trypsin :

a) Optimum pH = about 8.

b) Secreted as inactive form trypsinogen.

c) Trypsinogen is activated to trypsin by removal of a peptide of 6 amino acids at the N-terminus by two ways :

i) Enteropetidase (protease) secreted by the duodenum.

ii) Trypsin itself (autocatalytically).

d) It is an **endopeptidase** that acts essentially through serine residue **(serine protease)**.

e) It is specific for peptide bonds at the carboxyl groups of basic amino acids (Arg, Lys).

f) Products of digestion are **peptide fregments** (proteoses & peptones) and some free amino acids.

g) Trypsin inhibitor : Is a low molecular - weight peptide present in pancreatic juice that inhibits any trypsin formed in pancreatic cells when there is no need for it.

2) Chymotrypsin :

a) Optimum pH = about 8.0

b) Secreted as inactive form chymotrypsinogen.

c) Chymotrypsinogen is activated to chymotrypsin after removal of 6 amino acids at the middle of its peptide chain by trypsin.

d) It is an **endopeptidase** that act essentially through serine residue **(serine protease).**

e) It is specific for peptide bonds at the carboxyl group of arsomatic (Phe, Tyr, Trepto) and other (Met, leu) amino acids.

f) Products of digestion are like trypsin (peptide fragments).

3) Elastase :

a) Optimum pH = about 8.

b) Secreted as inactive form proelastase.

c) Proelastase is activated to elastase by trypsin.

d) It is an **endopeptidase** that act essentially through serine residue **(serine protease).**

e) It is specific for peptide bonds at carboxyl groups of aliphatic neutral amino acids (Ala. Gly, Ser).

f) Products of digestion are like trypsin (peptide fragments).

4) Carboxypeptidases :

a) Optimum pH = about 7.5

b) They are **metalloenzymes containing Zn^{2+}**.

c) Secreted as inactive form procarboxypeptidases which is activated by trypsin.

d) They are **exopeptidase** splitting amino acids from **the carboxyl end (C- terminal)** of peptide chain.

e) Specificity for peptide bonds :

i) Carboxypeptidase A : of aliphatic amino acids Val, leu, ILe, Ala.

ii) Carboxypeptidase B : of basic amino acids (Arg, Lys).

f) Products of digestion are **free amino acids** and **small peptides of 2-8 residues.**

5) Pancreatic lipase :

a) Optimum pH = 8.5.

b) It is activated in the intestine by a small peptide called **colipase** present in pancreatic juice that shift its pH from 8.5 to 6.5 which is the pH of intestine.

c) It is the major lipolytic enzyme (gastric and lingual lipases start lipid digestion in stomach & mouth, respectively).

d) Its activity at first is slow because it is **adsorbed** to the water-lipid interface (water-triacylglycerol) and converts triacylglycerols into fatty acids and diacylglycerols. These products possess polar and nonpolar groups that will adsorb to water - lipid interface in addition to bile salts and make hydrophilic surface on lipid droplets changing these droplets missible with water. This dispersion of lipids into smaller droplets **(emulsification)** will provide more sites for adsorption

of lipase molecules & more digestive action of it. This adsorption is also induced by the colipase.

e) It is specific for esters in positions 1 & 3 of triacylglycerols → free fatty acids and 2-monoglycerides (β-monoacylglycerol).

6) Phospholipases A_1, A_2 & C : They hydrolyze phospholipids.

a) Phospholipase A_1 : hydrolyzes the ester bond between FA and glycerol in position 1 → free FA + 2-acylglycerophosphoryl - base.

b) Phospholipase A_2 : hydrolyzes the ester bond between FA and glycerol in position 2 → free FA + Lysophospholipid.

c) Products of digestion of phospholipase A_1 & A_2 are **glycerophosphoryl - base + 2 FA.**

d) Phospholipase C hydrolyzes the bond in position 3 between glycerol and phosphate forming **1,2 - diacylglycerol + phosphoryl - base**.

7) Cholesterol esterase :

It **catalyzes** the hydrolysis of cholesteryl esters → **free choleseterol** → absorbed from the intestine.

8) Pancreatic amylase :

It is an **endosaccharidase** similar in mechanisms of action to salivary amylase but more active. End products of digestion are **maltose, maltotriose & α-limit dextrin** (oligosaccharide containing at least 8 glucose units with one or more α-1,6- glucosidic bonds).

9) Nucleases :

They include ribonucleases (RNase) and deoxyribonercleases DNase (that are responsible for hydrolysis of RNA & DNA into **oligonucleotides).**

C) Bile

1) Volume = 700 ml /day, pH = 7.7

2) Composition **(hepatic bile)**

a) Water = 98%

b) Solids = 2.0 %

- Inorganic (0.5%) : Na^+, Cl^-, HCO_3^- and little Ca^{2+}, K, HPO_4^{2-}, Zn^{2+}, Fe^{2+} & Cu^{2+}.
- Organic (1.5%) : bile salts, mucin, bilirubin, cholesterol, phospholipids and alkaline phosphatase.

3) Difference between **hepatic & gall bladder bile** :

Gall bladder bile differs from hepatic bile in :

- More **concentrated** (less water; 85%) which leads to increase in viscosity (more mucin), bile pigments, cholesterol, total solids and specific gravity.
- Aquire **greenish color** due to oxidation of bilirubin to biliverdin (green color).

4) Function :

a) Emulsification :

- Fat is immissible in water and there is a small intefacial area between them.
- Mechanical mixing due to intestinal movement (peristalsis) will brake fats into very small particles forming an **emulsion** with increased interfacial area between fat and water.
- Bile salts (sodium glyco- & taurocholate) will stabilize this fat emulsion due to its surface - tension lowering property (**detergent property**). Bile salts arrange themselves around lipid particles so that its nonpolar lipophilic part (steroid ring) dips into lipid

particle, and the polar hydrophilic part (carboxyl & hydroxyl groups of glycine & taurine) projects out towards the water phase. In this way lipid particles aquire negative charge and repel each other and form a **stable emulsion**.

b) Digestion of fat :

- Bile salts help emulsification of lipids with formation of **more sites for adsorption of lipase enzyme** to lipid particles. This will increase hydrolysis of fat in the intestine.

c) Absorption of fat :

- Bile salts in water aggregate into **micelles**.
- Bile salts are arranged in micelles so that the hydrophobic part of the of acid (steroid ring) are removed from contact with water while the hydrophylic part (carboxyl & hydroxyl groups) remain exposed to water.
- Fatty acids, monoacylglycerol, phospholipids & cholesterol are incorporated into bile acid micelles forming the intestinal **mixed micelles**.
- Mixed micelles carry lipids from the intestinal lumen to the cell surface where **transport occurs by diffusion** depending on the concentration gradient between lipid concentration in the lumen and that on the cell surface.
- Bile salt deficiency will lead to decreased fat absorption in the intestine and increased fat excretion in the stool (this is called **steatorrhoea**).

d) Neutralization of gastric juice acidity due to its content of bicarbonate buffer.

e) Inhibition of intestinal putrefaction :

Unabsorbed fat inhibits the digestion of protein which increases the intestinal bacterial putrifaction of proteins. Bile salts inhibit this process by increasing fat digestion and absorption.

f) Excretion :

Bile is the main route for excretion of **bile acids and cholesterol**. In addition several substances are excreted through bile such as drugs, toxins, bile pigments & inorganic substances as Zn^{2+}, Cu^{2+} & mercury.

g) Choleretic effect :

It is the increase of bile salt formation from the liver by the absorbed bile salts from the intestine.

D) Intestinal juice **(Succus entericus)** :

1) Volume : about 3 lit /day, pH = 7.7

2) Composition :

a) Water = 98.5%

b) Solids = 1.5 %

- Inorganic (0.9%): Na^+, cl^-, $HCO3^-$ and little K^+, Ca^{2+} & HPO^{2-}_4
- Organic (0.6%) : Lipids, mucin & enzymes.

E) Intestinal enzymes :

They complete the digestion of food.

1) Aminopeptidases :

- **Exopeptidases** that attack peptide bonds at N-terminus of the polypeptide. It is activated by Zn^{+3} or Mn^{2+} & present in the luminal surface of epithelial cells (**Brush border**).
- End products are **free amino acids & di- & tripeptides.**

2) Di- & Tripeptidases :

- They split di- & tripeptides into **free amino acids**.

- They are present **inside intestinal cells**.

3) Disaccharidases & Oligosaccharidases

- Present on the **surface membrane of intestinal cells**.
- Examples :
  a) Maltase : Hydrolyzes maltose into 2 glucose units (α-glucosidase).
  b) Sucrase : hydrolyzes sucrose into glucose & fructose,
  c) Lactase : hydrolyses lactose into glucose & galactose (β-glycosidase).
- Undigested di-, tri-, oligo - & polysaccharides cannot be absorbed and are utilized by the intestinal bacteria by anaerobic metabolic process. The resulting degradation products (H_2 gas, methane, CO_2, lactate) cause fluid secretion, increased intestinal motility and **cramps** due to increased intraluminal osmotic pressure & distension of the gut.
- Lactose intolerance : Is due to **deficiency of lactase**. Individuals deficient in lactase will not tolerate ingestion of milk and milk products.

4) Phosphatase :

It removes phosphate from certain organic phosphates as hexose phosphate, glycerophosphate, & nucleotides.

5) Nucleases :

As pancreatic nucleases.

6) Nucleosidases :

Split nucleosides into free nitrogen base + pentose phosphate.

7) Phsopholipases :

As pancreatic phospholipases.

ABSORPTION

General Considerations

A) Site of absorption :

- Stomach : little absorption.
- Small intestine : absorption of 90% digested food & water.
- Large intestine : absorb more water ⟶ more solid food residues.

B) Pathways for solute transport across epithelial cells of intestine:

- Transcellular : Through the cells (luminal & contraluminal plasma membrane).
- Paracellular : through the tight junctions between cells.

C) Function of gastrointestinal epithelial cells :

1) Luminal plasma membrane **(Brush border)** :
   - It is in contact with nutrients.
   - Specialized for terminal **digestion** of nutrients through its content of digestive enzymes (Disaccharidases, dipeptidases, ...)
   - Responsible for nutrient **absorption** through transport systems (for monosaccharides, amino acids, peptides & electrolytes) according to their concentration.

2) Contraluminal plasma membrane :
   - It is in contact with intercellular fluid, capillaries and lymph.
   - It possesses :

     a) **Receptors** for hromonal or neuronal regulation of cellular function.

     b) **Na^+, K^+- AT Pase** enzyme for pumping (removal) of Na+ out of cells.

c) **Transport systems** for entry of nutrients into cells then exist of these nutrients to blood so that the digestad food can become available to all body cells.

D) Absorption of Nacl :

- **Passive** entry of Na^+ & cl^- into cells across luminal plasma membrane of small intestine which is not affected by mineralocorticoid hormones.
- In the large intestine, Na^+ enters the cells by **Na^+ channel** present in luminal membrane down its concentration gradient. It is regulated by mineralocorticoid hormones.
- **Na^+ is removed from cells** by the **Na+, K+ - ATPase** present in contraluminal plasma membrane of cells (1 mole ATP is used to pump out 3 mole of Na^+ and inward pumping of 2^- mole K^+. This will maintain high K^+ and low Na^+ concentration inside cells.

E) Absorption of monosaccharides :

1) Glucose & galactose (Glc & Gal) :

- Glc & Gal have similar molecular configuration at C_2.
- The brush border (luminal plasma membrane) contains **a glucose transporter ($SGLT_1$)** which binds both D-glucose (1 mole) and Na^+ (2 mole) and transport them to inside cells.
- Na^+ is transported down its concentration gradient while glucose is transported against its concentration gradient.
- Absorption of glucose **requires free energy** from hydrolysis of ATP **linked to sodium pump** in which Na^+, K^+ ATPase expels 3 Na^+ outside cells in exchange for entry of $2K^+$ into cells.
- Sodium pump increases sodium concentration outside cells which allows more glucose absorption.

- Ouabain (cardiac glycoside) is a drug that inhibits glucose active transport by inhibiting sodium pump.
- Little glucose is passively absorbed using another glucose transporter **($GLUT_2$)** (in the contraluminal membrane) which is **dependent on glucose concentration.**

2) Fructose :

- Slowly absorbed **by diffusion** depending on its concentration in the lumen by Na^+ - independent transporter.

F) Absorption of Lipids :

1) The products of lipid digestion include :

a) 2-monoacylglycerol b) 1-monoacylglycerol.

c) Fatty acids (long chain & short chain).

2) These products diffuse into **mixed micelles and liposomes**, made of bile acids + phospholipids, and thereby can be incorporated, solubilized and transferred from emulsion droplets to the micelles.

3) As micelles are soluble, they carry products of lipid digestion to the aquous environment of the **brush border** of intestinal epithelial cells.

4) The following processes occurs in the intestinal cells :

a) 1- monoacylglycerol ⟶ glycerol + FF (by lipase).

b) 2- monoacylglycerol ⟶ triacylglycerols ⟶ adsorb phospholipids and apolipoprotein - A-1 & B ⟶ migrate as vesicles ⟶ fuse with plasma membrane ⟶ released into intercellular space ⟶ lymph vessels ⟶ chylomicrons ⟶ thoracic duct ⟶ systemic circulation. (Chylomicrons are milky fluid, the chyle, & consists of phospholipids, cholesteryl esters, triacylglycerols & fat soluble vitamins).

5) Short chain fatty acids (less than 10-12 carbons) are transported directly to the portal blood without passing to the lymph and without modification. These acids provide the liver with high caloric nutrient.

G) Absorption of proteins :

1) The products of protein digestion are amino acids and small peptides.

2) L-amino acids are transported against concentration gradient by two processes similar to those of glucose including **a) Na^+-dependent active transport system** in the brush border of cells **b) Na^+ - independent system** in the contraluminal membrane of cells.

3) Active transport is inhibited by 2, 4- dinitrophenol, the uncoupler of oxidative phosphorylation.

4) Dipeptides are transported by a transporter system and are hydrolysed in the intestinal membrane into amino acids.

5) Fetus & neonates small intestine can absorb **intact proteins**. This occurs by pinocytosis & is important for the **transfer of maternal antibodies to the fetus** ⟶ offsprings. If pinocytosis of large proteins remains in the adults life, **antibody formation** against these macromoleules will occur.

METABOLISM OF XENOBIOTICS

Definition :

Xenobiotics = exogenons substances which are foreign to life. Xenos = strange.

Examples of Xenobiotics :

1) Drugs.

2) Chemical carcinogens : Benzpyrene, aniline .... etc.

3) Environmental contaminants : Insecticides, polychlorinated biphenyls (PCBs).... etc that enter the body by food, air or contact with skin.

Geneneral Outline of Metabolism of Xenobiotics :

I) Metabolized mainly in the liver by certain enzymes.

II) Most of them are **lipophilic** and are **metablized into polar** (wath soluble) compounds which facilitate their excretion in urine or bile.

III) Metabolism of xenobiotics has 2 phases :

1) Phase 1 : Reactions catalyzed by enzyme species named **cytochrome P450** which catalyze mainly **hydroxylation reaction** in addition to deamination, desulfuration, epoxidation, peroxygenation & reduction reactions.

2) Phase 2 : The hydroxylased or other compounds produced in phase 1 are converted by other enzymes to various polar (water soluble) metabolities.This occur by :

a) **Conjugation** with glucuronic acid, sulfate, acetate, glutathione or certain amino acids.

b) **Methylation**.

D) Sometimes phase 1 reaction might convert xenobiotics from inactive to biologically active compounds like a procarcinogen or a prodrug are transformed to the carcinogen or the drug themselves.

Cytochrome P450 species :

A) Hemeprotein (like hemoglobin).

B) Present in all cells (except RBCs and muscle cells) in the cytosol (microsomes) and mitochondria (inner membrane). Liver cells are rich in P450 enzymes (constitute 20% of liver proteins).

C) Origin of its name (cytochrome P450) : Came from being a **pigment** with maximum absorption spectrums at wave length = 450 nm.

This was obtained when the microsomal fraction of cells was prepared, then it was reduced followed by exposure to carbon monoxide (CO) which binds to reduced heme protein and produces an **absorption spectrum with a peak at approximately 450 nm.**

D) Nomenclature of P450 gene subfamilies :

The term CYP, which represents the first two letters of cytochrome and the first letter in P450, is used as a prefece to designate a gene or a protein as a cytochrome P450.

E) The chief reaction catalyzed by Cytochrome P450 is hydroxylation and the enzyme is a **monoxygenase** because only one of the 2 oxygen atoms of O_2 is incorporated into the substrate and the other atom unites with H of NADPH to form water. Therefore the reaction is written as follows :

$$\mathbf{R\,H} + O_2 + \text{NADPH} + H^+ \rightarrow \mathbf{R\text{-}OH} + \text{NADP} + H_2O\,.$$

Where RH = substrate.

F) Mechanism by which O atom is incorporated into substrate :

- Cytochrome P450 proteins contain :

a) Single iron protoporphyrin IX prosthetic groups which binds oxygen.

b) Substrate binding site.

- First : The cytochrome P450 binds a substrate. This will accelerate reduction of cytochrome P450 by electrons given by NADPH through **NADPH – Cytochrome P450 reductase enzyme**.
- Second : Electrons are transferred from reduced cytochrome P450 to Fe^{3+} of heme → ferrous Fe^{2+}.

  This will allow oxygen to bind to heme iron.
- Third : Oxygen is cleaved from cytochrome P 450 to generate the active oxygen species for insertion into reaction site of the substrate → Hydroxylation.

G) Genes of cytochrome P450 species **(gene superfamily)** : Over 300 cytochrome P450 genes, coding for different proteins catalyzing oxygenation of substrates, had been characterized. Each enzyme has a specific substrate.

H) Physiological function of cytochrome P450 :

- It metabolize a variety of **lipophilic compounds** of endogenous or exogenous origin. This occurs by **simple hydroxylation,** hydroxylation of aromatic ring to form **phenols** or addition of an oxygen atom across a double bond to form an **epoxide**.

  In addition they catalyze oxidation of sulfur, phosphorus and nitrogen atoms and dehalogenation reactions.
- Substrates include :
  - Synthesis of steroid hormones from **cholesterol** in adrenel cortex & sex organs. This occurs by **cleavage of the side chain of cholesterol** through sequential **hydroxylation**

reactions in the side chain by cytochrome P450 producing pregnenolone which is the precursor of steroid hormones (cortisol, progesterone, aldosterone, androstenedione).

- Synthesis of estrogens from adrogens by an **aromatization reaction** (because an aromatic ring is introduced into the product) using a **single cytochrome P450 (aromatase)** which catalyzes multiple hydroxylation reactions to form the aromatic ring and remove the methyl group at C_{19} of testosterone → estrogens.
- Change Vit D_3 to 1,25 dihydroxy Vit D3 (active form of Vit D_3).

1) Exogenous substances :

- These are often referred to as **xenobiotics**.
- Examples (as before).
- Change them from being lipophilic → water soluble → excreted → prevent their storage in cells. (many xenobiotics interfer with cell function).
- Sometimes cytochrome P 450 system is responsible for generation of carcinogens. But cancer does not occur because other factors will prevent this like the immune system, nutritional state, genetic predisposition & environmental factors.
- Therefor cytochrome P450 plays a signifciant role in health and disease.

I) Induction of cytochrome P450 :

- Various cytochromes P450 can be induced by both endogenous & exogenous compounds.
- Many drugs (phenobarbitone ... etc), PCBs or hydrocarbons induce cyt. P450.

- Mechanism of induction include :

  Drug → enter the cytosol → receptor → nucleus → bind to regulatory regions of **cytochrome P450 genes (response element)** → increased transcription of mRNA from DNA → increased protein biosynthesis of Cyt. P450 protein enzymes.

J) Polymorphism of cyto. P450 genes :

Individuals may differ in their rates of metabolism of a particular drug because **differences in the cytochrome P450 gene may exist in a given population**. This genetic polymorphism cause an individual to be unable to metabolize a drug at a sufficient rate, thereby producing elevated drug levels → **drug toxicity**.

Phase 2 metabolism of xenobiotics :

Phase 1 reactions generally produce hydroxylated polar derivatives which are conjugated in phase 2 reaction → more water soluble → excreted in urine or bile

1) Glucuronidation : The most frequent.
   - Similar to glucuronidation of bilirubin in the liver.
   - UDP - glucuronic acid (UDP = uridine diphosphate) is the glucuronyl donor & glucuronidation reactions are catalyzed by **glucuronyl transferases** (in the endoplasmic reticulum).
   - Substrates include : carcinogens, aniline, benzoic acid, drugs, phenol and many steroids.
2) Sulfation :
   - Sultate donor = Adenosine 3′- phosphate - 5′ - phosphosulfate **(PAPS)** or active sulfate.
   - Substrates include : some alcohols, phenols & arylamines.
3) Conjugation with glutathione (GSH) :

- Glutathione is a tripeptide consisting of glutamic acid, cysteine & glycine.
- Abbreviated as GSH (SH indicates the sulfhydryl group of cysteine and is the active part of GSH).
- **Glutathione S transferase enzyme** is present in the **liver cytosol** and catalyzes conjugation of xenobiotics (R) with GSH as follows :

$$R + GSH \xrightarrow{\text{transferase}} R - S - G$$

- Glutathione conjugates are further metabolized before excretion. The glutamyl & glycinyl groups are removed & an acetyl group (acetyl COA) is added to the amino group of cysteine → mercapturic acid → excreted in urine.
- Other functions : Due to its - SH group:
  a) Removal of $H_2 O_2$ (toxic to cells).
  b) Act as reducing agent helping to maintain SH group of enzymes in their reduced state → prevent enzyme inactivation.
  c) Transport of certain amino acids across membranes of the kidney by γ-**glutamyl trnasferase enzyme**.

4) Acetylation

R + acetyl - COA → acetyl- R + COA

- Catalyzed by **acetyltransferase** present in liver cytosol.
- The drug isoniazide (for treatment of tuberculosis) is acetylated before excreted.

5) Methylation

- Methyl donor is **S-adenosylmethionine**.
- Catalyzed by **methyl transferase**.

Responses to Xenobiotics :

1) Pharmacologic response :
   - Phase 1 reactions might produce the active form of the drug.
   - Pharmacologically active drugs act without being metabolized by cyt. P 450 . However cyt. P 450 deminish or terminate the action of these drugs by hydroxylating them → easily excreted.
2) Cell injury
   - May result in cell death.
   - Xenbiotics bind by covalent bonds to cell macromolecules like DNA, RNA & proteins → severe effect on cellular function as oxidative phosphorylation & regulation of the permeability of plasma membrane → cell injury.
3) Immunologic damage :

   Xenobiotics may bind to proteins → modify & **alter its antiginicity** → **antibodies** against body cells → cell damage.
4) Chemical carcinogenesis :
   - Some chemicals like benzpyrene → hydroxylated → carcinogenic products (indirect carcinogens).
   - Some monoxygenases **transform procarcinogens into epoxides** which are highly carcinogenic.
   - Epoxides are converted to less reactive compounds (dihydrodiol) by **epoxide hydrolase enzyme** present in the endoplasmic reticulum.

Bibliography

1) **Cantarow, A & Schepartz, B (Eds)** : Biochemistry. Fourth edition, Saunders Company, Philadelphia and London, 1967.

2) **Champe, PC & Harvey, RA (Eds)** : Lippincott's illustrated review : Biochemistry. Lippincott Company, Philadelphia, 1987.

3) **Devlin, TM (Ed)** : Textbook of biochemistry with clinical correlations. Fourth, edition, Willey & Sons, Inc., Publication, New York, 1997.

4) **Marks, DB (Ed)** : Biochemistry (Board review series). Williams & Wilkins, Baltimore, 1992.

5) **Murray, R.K, Granner, D K, Mayes, PA and Rodwell, VW (Eds)** : Harper's Biochemistry. 25th edition, Appelton & Lange, Connecticut and California, 1999.

6) **Schumm, DE** : Essentials of Bicohemistry. In : D.T. Lowenthal (Ed), Essentials of Medicial Education Series, Davis Company, Philadelphia, 1988.